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THE PATHOLOGIC ANATOMY OF SPLENOMEGALY*

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The object of pathologic anatomy is to classify morbid entities on the basis of morphologic investigations, and to coördinate structural alterations with functional changes. It must be admitted that at present these ideal aims are not always accomplished. In the spleen, this approach is even more difficult, because of the complexity of its morphology and the inadequate knowledge of its function. Furthermore, the question arises as to whether there really do exist diseases in which a splenic alteration is the primary or initiating factor. Since the spleen is more or less involved in so many fundamentally different morbid states, one must consider the possible secondary nature of all alterations of this organ. Added experience has shown that the spleen is not the sole organ affected, even in conditions in which the splenic alteration is most conspicuous. This is clearly seen in myeloid leukemia, which was originally regarded as splenic leukemia until Neumann demonstrated a simultaneous alteration of the bone marrow. The splenomegaly in Gaucher's disease was originally regarded as primary, but is now recognized to be part of a systemic disease. One may justly maintain that the spleen participates in all pathological changes of the mesenchyme, because it is nothing more than mesenchyme which has been concentrated in the form of an organ. Morbid states of the mesenchyme may occasionally manifest themselves within the spleen so prominently, as to give the impression of being primary splenic disease. These considerations may lead to a view point that only the traumatic or topographic alterations, neoplasms, and cysts can, with certainty, be regarded as primary disorders of the spleen.

* Read at a Symposium on Tumors of the Hematopoietic System, held in New York City under the auspices of the American Society of Clinical Pathologists, June 4, 1935.

Nevertheless, it is desirable to classify all the morbid conditions of the spleen in which enlargement is a conspicuous feature. Many authors (Lubarsch, Naegeli, Morawitz) have tried to divide the various forms of splenomegaly into well-defined groups. Their attempts at classification, however, were not based upon consistent principles, because they utilized both clinical and pathological criteria. These classifications are found to be erroneous in that they accept indefinite clinical syndromes, (Banti's disease and splenogenous anemias) as definite morbid entities. Increasing experience has shown that these vague diagnoses are applied to conditions of varied pathogenesis, and should be abolished rather than retained. Furthermore, these classifications unite under one heading "splenomegaly in blood dyscrasias" such morbid entities as hemolytic icterus, thrombopenic purpura, and leukemia, even though these diseases have undoubtedly neither morphologic nor pathogenic association.

Strasser, proposed a classification based on teleologic principles. However, our knowledge is not as yet sufficiently advanced to permit the application of these principles for purposes of classification. For instance, the classification of the splenomegaly in Banti's disease among the group of "chronic defensive hyperplasias" is inadequate. The splenic enlargement in hepatic cirrhosis is regarded as compensatory hyperplasia secondary to destruction of "extra-splenic reticulo-endothelial tissue." This view-point will find as little acceptance as the idea originally presented by Grawitz and Hartwich, that the splenomegaly in cirrhosis is designed to compensate for the loss of liver tissue. The insertion of the splenomegaly of hemolytic icterus among "pulpous-constitutional hyperplasias" is meaningless, and the thrombophlebitic splenomegaly has been entirely omitted.

A purely descriptive classification, as suggested by Fox and McCarthy has never been practically applied and it is questionable whether it could satisfy the demands of the clinician.

A strict morphologic-pathogenetic classification based upon an analysis of the structural alterations in the spleen might best serve our needs in the diagnosis of splenomegalies. A thorough knowledge of the normal histology of this organ is therefore essential, but, space does not permit too detailed a description.

The architecture of the spleen can best be understood if one considers the fundamental framework of the organ, which consists of reticulum cells (the cytoplasmic reticulum) and fibers (fibrillar reticulum). Mollier has described how the finer vascular channels in the spleen develop from the reticulum by a process of canalization, and how they are interwoven into this basic framework of the organ. The meshes of the framework contain the free cells of the spleen, which are lymphocytic, granulocytic, and histiocytic in nature. The cytoplasmic reticulum is an undifferentiated mesenchymal tissue (Klemperer, lit.) which with adequate stimuli is capable of differentiation into any one of the free elements.

Hueck (1929) divides the splenomegalies morphogenetically into three groups:

1. Alterations in the size of the reticulum meshes due to (a) edema; (b) arterial hyperemia; (c) venous hyperemia.

2. Alterations of the reticulum due to (a) infiltrations (storage) of foreign substances in the reticulum cells and their progeny (macrophages); (b) degeneration (amyloid or hyalin) of the ground substance about the fibrillar reticulum.

3. Proliferation (hyperplasia) of the reticulum and its derivatives.

The last group includes most of the chronic forms of splenomegaly.

This strictly morphologic classification is too general for practical clinical purposes. To meet the requirements of diagnosis, pathogenetic aspects must also be considered. The forms of splenomegaly can be tentatively classified, as in the case of enlargement of any other organ, into five groups. Enlargement of organs, in general, are the result of one or more of the following factors; (1) inflammation; (2) infiltration (e.g., fatty liver); (3) hyperplasia (increase in the number of constituent elements); (4) neoplasms; (5) cysts.

In attempting to classify all the forms of splenomegaly according to this scheme, difficulties are encountered. First, as will be discussed in a subsequent paragraph, not all known types of splenomegaly can be included. Secondly, one and the same form

sometimes belongs to two different groups. In such instances, the classification is made according to the more conspicuous lesion, or the more restrictive etiologic criteria. Two examples may illustrate this point. In Gaucher's disease, the splenomegaly is undoubtedly due, not only to the storage of kerasin, but also to hyperplasia of the fixed and free macrophages. Nevertheless, this form is included among the infiltrative splenomegalies, because this is the more characteristic histologic feature. In chronic inflammatory splenic tumors there is a proliferation of cells and fibers of the original reticulum. From a strictly morphologic viewpoint one could regard such instances as hyperplastic conditions. However, the pathogenetic factor differentiates the tissue increase here from that of other forms of which the origin is unknown; therefore, splenomegaly caused by inflammatory hyperplasia is classified with inflammation.

It appears advisable to at first discuss the characteristic morphologic features of each group. In the second part of this paper an attempt will be made to apply these fundamental criteria to those forms of splenomegaly which have so far been classified according to their clinical aspects only.

1. SPLENOMEGALY DUE TO INFLAMMATION

The enlargement of the spleen in acute infections, usually called "acute infectious splenic tumor," does not generally reach dimensions sufficient to justify the designation splenomegaly. The size is dependent upon alterations in the volume of blood within the spleen (active hyperemia), the edema and the increase of the cellular contents. According to Botkin, (quoted by Jawein) and Litten, the active hyperemia is caused by paralysis of the vasoconstrictor nerves; according to Hueck, 1929, it is the result of irritation of the vasodilators. Insufficient attention has been paid to the occurrence of edema within the human spleen. Lubarsch (1927) applies the term "parenchymatous edema" to marked dilatation of the meshes of the pulp in which there are few erythrocytes and leukocytes. He casts some doubt upon the intra-vital origin of this change. However, Roessle (1928) and Hueck (1929) believe in the existence of an intra-vital accumula-

tion of plasma within the meshes of the pulp; according to the former, this occurs in infections and in post-hemorrhagic swelling of the spleen.

The cellular increase is due to the exudation of erythrocytes and leukocytes together with the proliferation and mobilization of fixed elements associated with storage of fat and iron, and phagocytosis of injured cells, foreign elements (bacteria), and tissue débris. The changes which can be observed in the morphological picture of acute infectious splenic tumor depend upon complex variations of these component factors. The appearance is further modified by subsequent degenerative alterations of the fixed tissue elements and of the emigrated and proliferated free cells. All of these morphologic changes are regarded as a reaction of the spleen to the circulating infectious agents or toxins, and therefore justify the term "acute splenitis."

Conspicuous proliferation of the histiocytic cells with marked erythrophagocytosis characterizes the acute splenic swelling seen in diseases of the typhoid-paratyphoid group. In infections caused by pyogenic cocci, exudation with abundant granulocytes and plasma cells predominate. Evans distinguishes a red splenic tumor in typhoid and a gray enlargement in coccal infections. In other acute infections, such as diphtheria, malleus, tularemia, certain definite morphologic features are frequently encountered which permit one to at least suspect their etiology. I refer to the hyperplasia of the secondary follicles in the first, and necrotic foci in the latter two diseases (Lubarsch, Francis). Conspicuous histiocytic reactions occur in acute malaria and other protozoal diseases. The parasites and their metabolic products (pigment) are found within the enlarged elements of the reticulo-endothelial system. The recognition of the organisms in sections clinches the diagnosis, even though the histologic findings alone are not sufficiently characteristic.

The histological alterations of the spleen in infections are determined only in part by the specificity of the exciting agent, and to a larger extent governed by variations in the response of the splenic mesenchyme. This is apparent by comparing the macroscopic and microscopic changes of the spleen in acute and chronic

streptococcal infections. In the latter, as in *subacute bacterial endocarditis*, the splenic enlargement is frequently so great that it can justly be designated as splenomegaly. The organ is much firmer and appears less cellular than in the acute infectious swelling. The malpighian corpuscles are often very large.

Microscopically the large splenic corpuscles show conspicuous secondary follicles with proliferation of reticulum cells. They

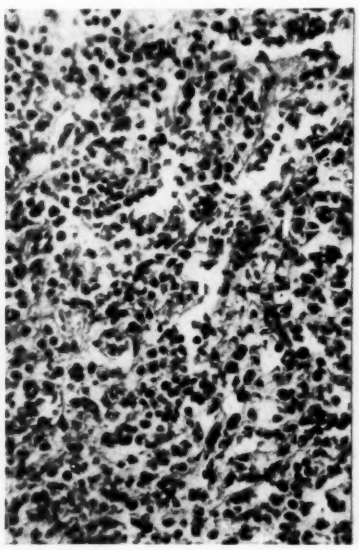


FIG. 1

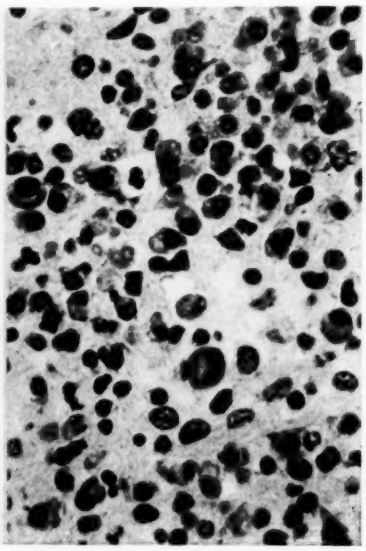


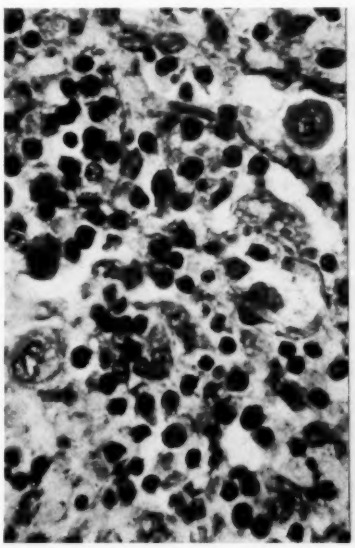
FIG. 2

FIG. 1. SUBACUTE STREPTOCOCCUS VIRIDANS ENDOCARDITIS
Widening of Billroth's cords due to cellular infiltration

FIG. 2. SUBACUTE STREPTOCOCCUS VIRIDANS ENDOCARDITIS
A nest of plasma cells within the red pulp

often show phagocytosis of nuclear débris. Frequently the reticulum also show karyorrhexis and occasionally the entire center of the follicle is necrotic. Within the red pulp, granulocytes are less numerous than lymphocytes and plasma cells and free histiocytes are characteristically conspicuous within the widened Billroth's intersinusoidal cords (figs. 1 and 2). These latter cells frequently, though not always, show vacuoles (fig. 3), and en-

gulfed erythrocytes and leukocytes and blood pigment. The cytoplasmic reticulum is predominantly stimulated, but the sinus endothelium may also proliferate (Schilling, 1919). Scattered myelocytes and small groups of hemocytoblasts are occasionally encountered within the sinuses and cords. The small arteries of the pulp are surrounded by plasma cells and their adventitia is



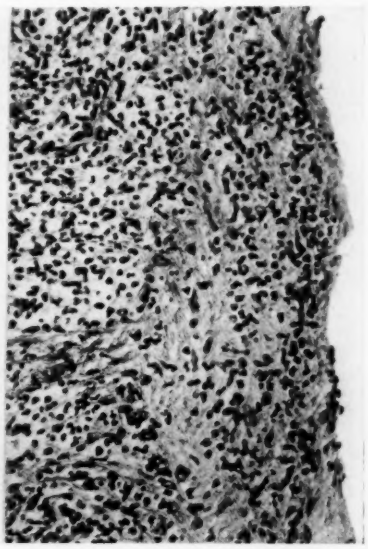


FIG. 3 FIG. 4

FIG. 3. SUBACUTE STREPTOCOCCUS VIRIDANS ENDOCARDITIS
Macrophages within Billroth's cords and sinuses

FIG. 4. SUBACUTE ENTEROCOCCUS ENDOCARDITIS
Capsular and subcapsular leucocytic infiltration. Necrobiosis of reticulum cells.

frequently thickened. In addition there is either focal or diffuse fibrosis of the fibrillar reticulum about the perifollicular zones of the red pulp (fig. 6). I have observed frequently subcapsular (fig. 4) and peritrabecular (fig. 5) foci of necrobiosis of the reticulum. This is probably identical with the lesions described by Fox as resembling Bracht-Waechter nodules of the myocardium. From a morphological point of view this connective tissue increase

represents the cicatricial or terminal stage of the earlier acute splenitis, but from a biologic basis, it may be regarded as a stage of exhaustion of the reacting splenic reticulum. Morphogenetically, then, the splenomegaly in chronic infections is accounted for by the general increase in the cellular content and by a more or less advanced new formation of collagen fibers. Because of the residual focal infiltrations by plasma cells and leukocytes, the

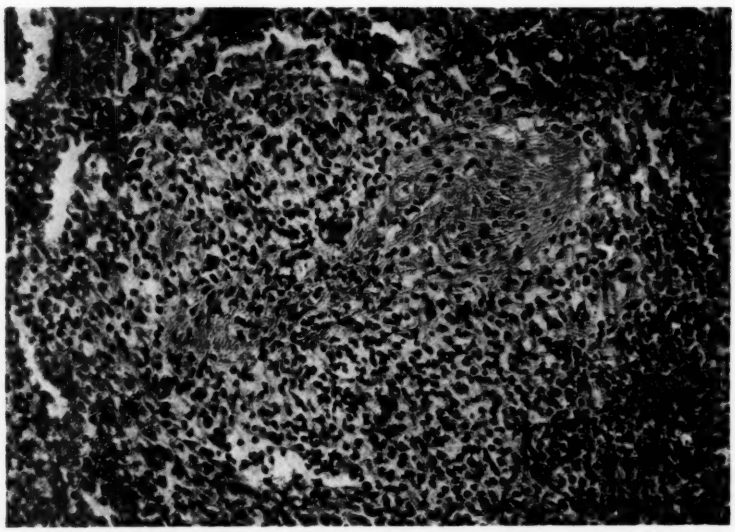


FIG. 5. SUBACUTE ENTEROCOCCUS ENDOCARDITIS

Trabecular and peritrabecular leucocytic infiltration. Necrobiosis of reticulum cells.

inflammatory origin of such alterations is readily recognized even without knowledge of the clinical picture.

The chronic splenomegaly of *protozoal origin* shows both histiocytic proliferation (fig. 7) and focal or diffuse fibrosis of the pulp. The causative agent is usually easily determined by the presence of parasites (fig. 8) or of the characteristic pigment within macrophages. However, in chronic malaria one occasionally encounters an enormous splenic enlargement with advanced fibrosis. In these instances in which the inflammatory

cellular increase can no longer be recognized, and the malarial pigment has disappeared, the etiology becomes exceedingly difficult to recognize (cf. plate 10, fig. 2, in Marchiafava and Bignami).

In *tuberculosis* (Winternitz, lit.), the spleen is rarely sufficiently large to be designated splenomegaly. Even the innumerable tubercles which riddle the spleen in generalized miliary tuberculosis do not produce a conspicuous splenic tumor; the moderate

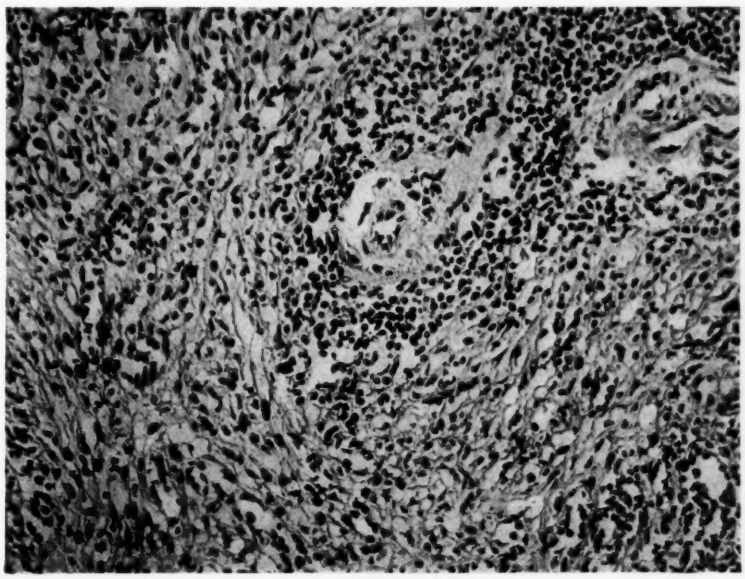


FIG. 6. SUBACUTE BACTERIAL ENDOCARDITIS (BLOOD CULTURE REPEATEDLY NEGATIVE)
Perifollicular fibrosis

enlargement is in a large measure caused by the concomitant active hyperemia and the additional non-specific cellular exudation and proliferation.

In actual splenomegaly due to tuberculosis, the weight may reach four kilograms, and the gross appearance is that of multiple coarse large caseous nodules, or that of conglomerated epithelioid tubercles (Leidel). The latter cases often run a prolonged course, and the bacilli are probably of low virulence (Goebell-Hasenbein;

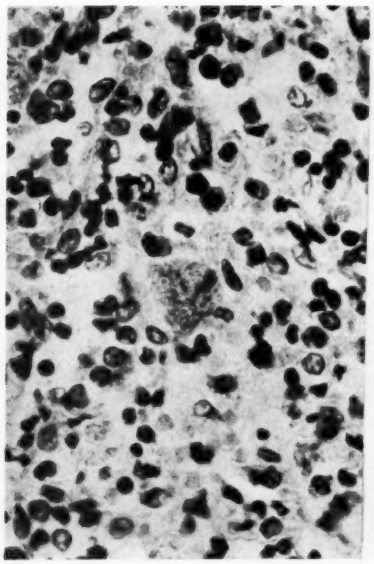


FIG. 7

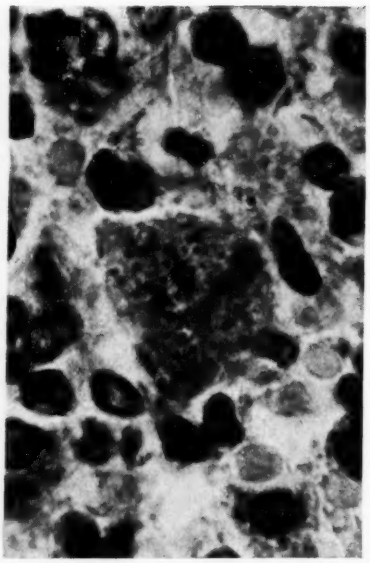


FIG. 8

FIG. 7. KALA-AZAR

Predominance of histiocytes with very large macrophages

FIG. 8. KALA-AZAR

Higher power of figure 7. Macrophage with numerous Leishman-Donovan bodies.

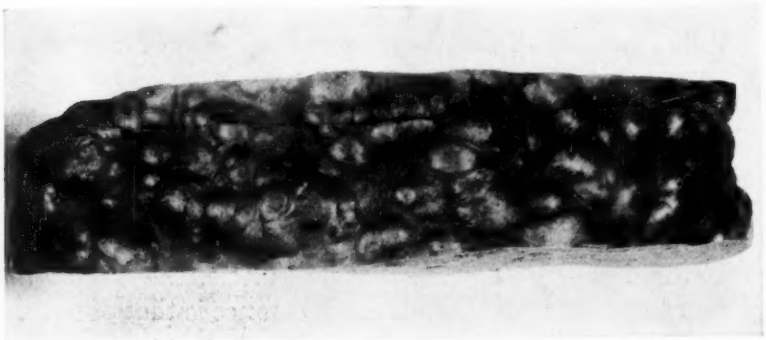


FIG. 9. COARSE NODULAR TUBERCULOSIS OF SPLEEN

Lubarsch, 1927). I have observed two cases which are apparently of this type; the surfaces of these very large spleens were studded with prominent cherry-sized nodes (fig. 9).

In one of these cases (published by R. F. Carter) diffuse lymphadenopathy was also present. Microscopically, the lymph nodes and spleen showed diffuse infiltration with epithelioid tubercles (fig. 10). Guinea pig inoculations with the glandular material evoked no response. Hence, an absolute conclusion as to etiology could not be reached, although the histologic features seemed characteristic of tuberculosis.

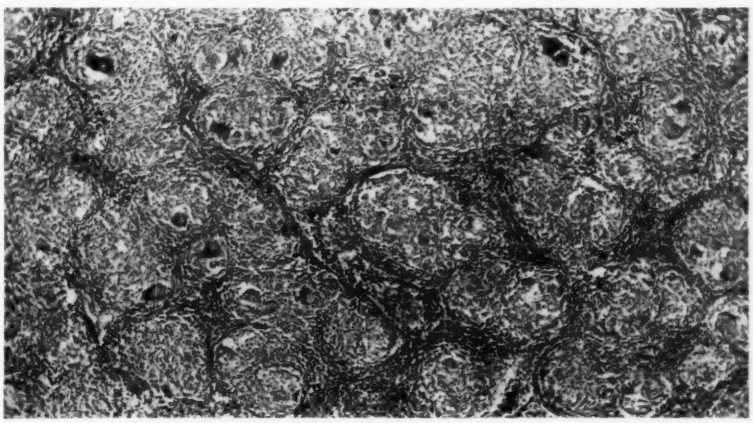
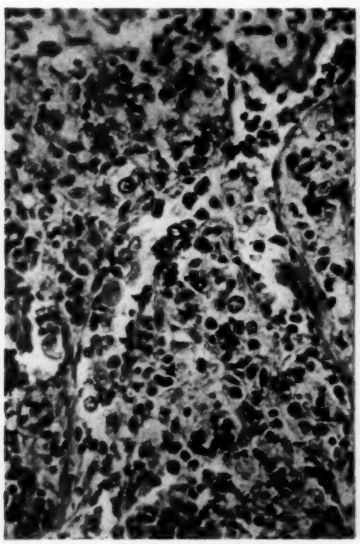


FIG. 10. HISTOLOGIC APPEARANCE OF FIGURE 9
Nodules composed of epithelioid cell tubercles

Splenic enlargements occur in both *congenital* and *acquired syphilis*. Congenitally luetic infants usually have a very firm, large spleen (Birch-Hirschfeld; Lubarsch, 1927). The splenic tumor is caused to some extent by the vascular stasis due to the interstitial hepatitis; and in part by the hyperplasia of the reticulum cells of the red pulp which show conspicuous erythro- and siderophagocytosis (Watson). There are, in addition, many macrophages containing lipoid material (fig. 11) areas of hematopoiesis, (fig. 12) and a thickened fibrillar reticulum. Perivascular plasma cell infiltration and many leucocytes also contribute to

the inflammatory picture. Occasionally follicles with epithelioid cells containing lipoid material (Beer's folliculoid hyperplasia) are seen. Typical syphilitic vascular changes or gummata are rare.

In the tertiary stage of acquired syphilis, very large gummata may occur, but these are unusual. I have observed a case of this



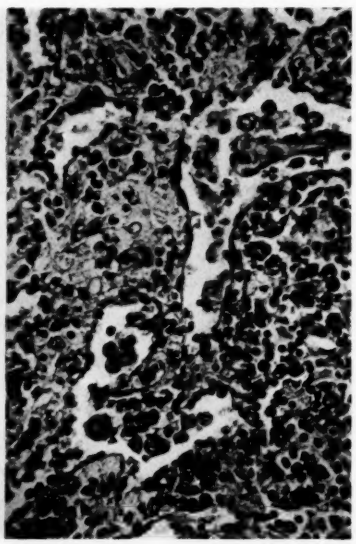


FIG. 11 FIG. 12

FIG. 11. CONGENITAL SYPHILIS

Widening of Billroth's cords by numerous macrophages. Same cells within the sinuses.

FIG. 12. CONGENITAL SYPHILIS

Similar appearance with nests of hemocytoblasts within the sinuses

type in which the enlarged organ was removed (fig. 13). The anemia in this case pointed to a clinical diagnosis of splenic anemia.

Diffuse enlargement without gummata is occasionally observed in acquired syphilis. It may be secondary to hepar lobatum, presenting the histological splenic changes occurring in cases of hepatic cirrhosis, or it may be due to an associated amyloidosis.

The splenomegaly in *schistosomiasis* (Nishikawa; Hutchinson), is usually attributed to portal stasis dependent upon the inter-

FIG. 13. GUMMA OF THE SPLEEN

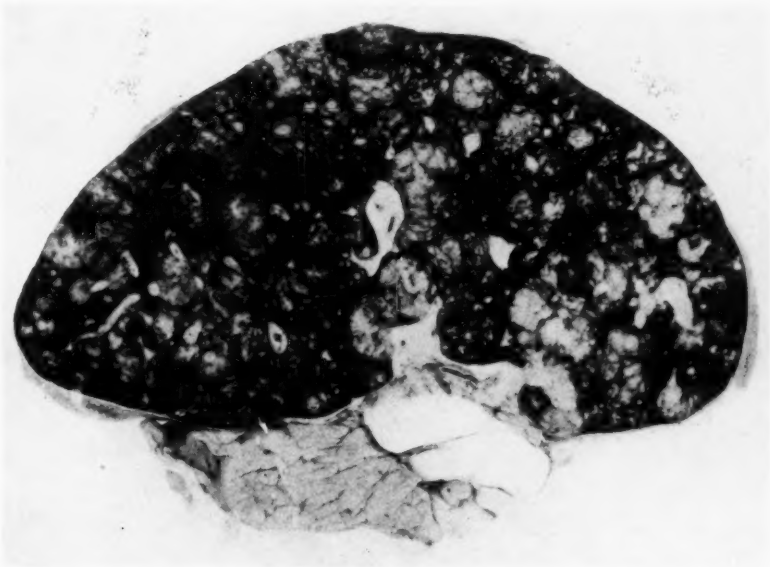
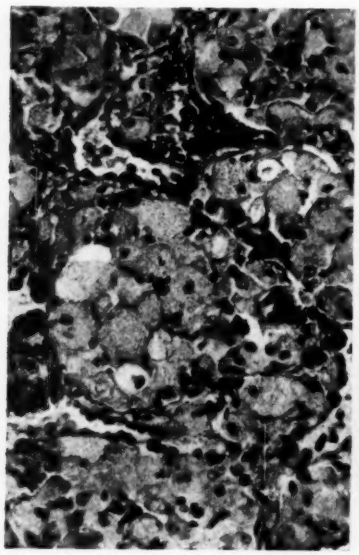


FIG. 14. HODGKIN'S DISEASE. PORPHYRY SPLEEN

stitial hepatitis caused by the bilharzia. The toxic products of the parasites, in addition, cause inflammatory changes in the spleen which might often lead to excessive splenic enlargement.

Histologically the enlargement of the spleen is due to hyperplasia of the reticulum cells and infiltration of the widened Billroth's cords by polymorphonuclear leukocytes and plasma cells. The number of sinuses is often increased especially adjacent to trabeculae. There are periarterial hemorrhages adjacent to fol-



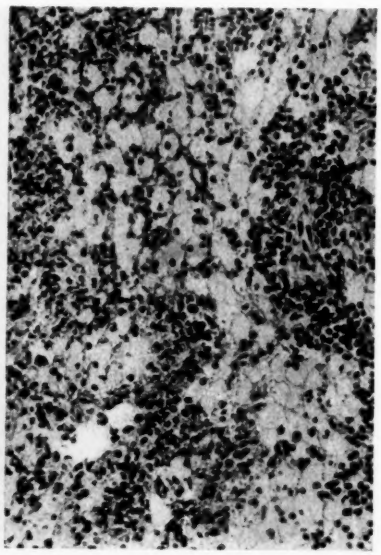


FIG. 15 FIG. 16

FIG. 15. GAUCHER'S DISEASE

Nests of Gaucher cells of reticulum cell origin. Sinus endothelium not affected.

FIG. 16. NIEMANN-PICK'S DISEASE

Nests of large vacuolated macrophages

licles. The histologic appearance very much resembles that found in hepatic cirrhosis.

The enlargement of the spleen in *Hodgkin's disease* will also be introduced with this group of inflammatory splenomegalies, even though the inflammatory nature of the disease has not been generally accepted. The lymphogranulomatous nodules frequently found within the spleen need not necessarily cause con-

spicuous enlargement, but there are cases in which the splenomegaly equals even that of myeloid leukemia. The characteristic macroscopic (fig. 14) and microscopic findings in this disease permit a diagnosis to be made with ease in a majority of cases. This is of importance in those rare instances in which Hodgkin's disease is limited to the spleen. Recently, Torre and Jona have described splenomegaly in generalized Hodgkin's disease in which the spleen did not show nodular infiltrations. The microscopic appearance was that of a non-specific inflammation. I have observed one similar instance (case 8, Moolten).

2. SPLENOMEGALY DUE TO INFILTRATION

Excessive storage of normal or abnormal metabolic products can result in enlargement of an organ. In the spleen it is necessary to distinguish between infiltration of the cytoplasmic reticulum and of the fibrillar reticulum.

Infiltration of the fixed reticulum cells and their mobilized descendants with lipoids characterizes the splenic enlargement of Gaucher's (fig. 15) and Niemann-Pick's disease (figs. 16, 17) and certain cases of diabetic lipemia. In these instances, however, the enlargement is due, not only to lipoid infiltration of the histiocytes analogous to that seen in the infiltrated fatty liver, but also to an increase in the number of these storing cells (hyperplasia).* Nevertheless it seems appropriate to classify the splenomegaly of Gaucher's disease and allied conditions in the group of infiltrative enlargements because of the predominating feature of the histologic picture. These features have been so completely discussed by numerous writers that a description is omitted here (Pick, Bloom and others).

A large and very firm splenic tumor occurs in amyloid disease, particularly in diffuse amyloidosis of the pulp. The significant relationship of this to chronic suppuration, tuberculosis, syphilis, Hodgkin's disease and malignant tumors, is generally known.

* It is possible that the pathogenesis of Gaucher's disease consists of a primary hyperplasia of the reticulum cells with a secondary disturbance of fat metabolism—and not, as is generally believed, in a primary disturbance of lipoid metabolism with secondary reticulum cell proliferation.

However, there are some cases in which neither the clinical nor even the anatomical investigations yield a clue as to etiology. Such cases offer great diagnostic difficulties during the life of the patient.

The amyloid substance is deposited within the ground substance adjacent to the reticulum fiber (Hueck, 1929). The fiber does not undergo primary degeneration, but it may later atrophy and disappear because of pressure (Ebert). The selective localization

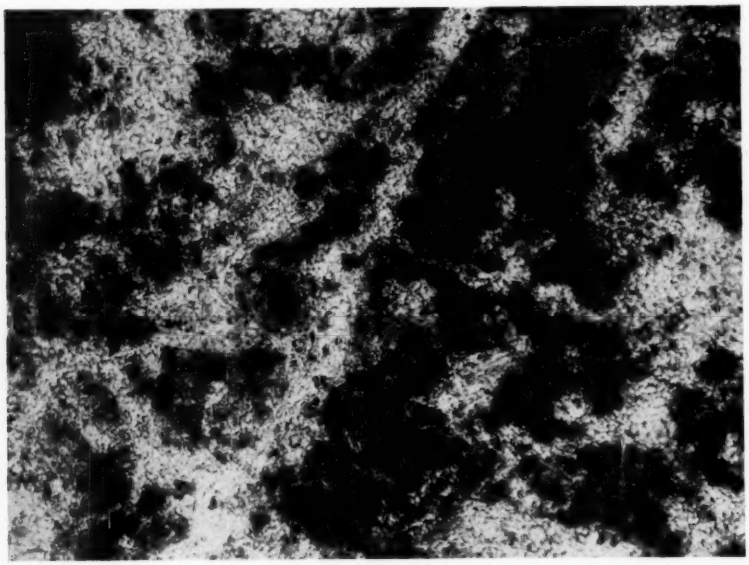


FIG. 17. NIEMANN-PICK'S DISEASE
Lipoid in cells stained black by Smith-Dietrich's method

of the amyloid either within the splenic follicles (sago spleen), or within the red pulp (ham spleen), is dependent upon the presence or absence of a follicular vascular network (Hueck, 1927; Jaeger, 1929). This network occurs more frequently in young individuals. This fact accounts for the prevalence of follicular amyloidosis among the young. Lubarsch (1927) (2), on the other hand, maintains that the localization of amyloid deposits also depends upon the type of primary disease. He found the sago

distribution mainly in tuberculosis and Hodgkin's disease, and the diffuse form in chronic suppuration, syphilis, and neoplasms.

3. SPLENOMEGALY DUE TO HYPERPLASIA

The increase in the size of an organ caused by an increase in the number of its constituent elements is designated as hyperplastic enlargement.

A study of the embryonal development of the spleen shows that the organ develops from a primordial cellular reticulum which persists in adult life, and, as cytoplasmic reticulum, represents an important constituent of the fully developed spleen. For this reason enlargements which are the result of proliferation of this embryonal tissue belong in the group of hyperplastic splenomegaly.

The cytoplasmic reticulum may, although it rarely does, proliferate in its undifferentiated form. In such cases, the splenomegaly is only part of a generalized reticulosis as in the case of Tschischtowitsch and Bykowa. In most instances of hyperplasia, however, the proliferating cells belong to one of the differentiated forms mentioned above, either of histiocytic or hematic type. Inflammatory stimuli may call forth an excessive proliferation of the reticulum cells, and its progeny, the mobilized histiocytes. This type of chronic hyperplastic splenitis may exist for years without clinical evidence of infection. It is the inflammatory background of plasma cells and polymorphonuclear leukocytes in these instances which discloses the pathogenesis of the reticulum hyperplasia and places them into the inflammatory group.

The hyperplasia of the histiocytic reticulum caused by disturbances of lipoid metabolism can likewise be easily recognized from its associated histologic picture. These instances are, however, grouped with the infiltrative splenomegalies for the reasons stated above.

Reticulum hyperplasia can also be provoked by stimuli the nature of which cannot be so readily recognized from the histologic appearance of the enlarged spleen. For example, local stasis in the spleen, as will be discussed in detail in a subsequent paragraph, is often associated with considerable enlargement because

of reticulum hyperplasia. In these instances, certain co-existing histological criteria, such as sinus hyperplasia, point to the etiologic factor.

The influence of the thyroid upon the size of the spleen is evident from the experiments of Tietze, who found that mice fed on thyroid gland showed conspicuous splenic enlargement due to an increase in the lymphatic tissue. The frequent splenic enlargement in Grave's disease is probably of a similar nature. One must consider the possibility that hyperplastic splenomegaly can be caused to some extent by hormonal stimuli. Muehlmann believes that certain cases of constitutional splenomegaly may be due to a disturbance of the vegetative nervous system. It is possible that these might also belong to the hyperplastic splenomegalies.

There is no doubt that splenic enlargement due to excessive blood cell formation is to be included with the group of hyperplastic splenomegaly. It is generally recognized that the hematic cells develop locally within the spleen from pre-existing stem cells and from the multipotent cytoplasmic reticulum (Lang, Klemperer, lit.).

Mention must first be made of the splenic enlargement in *severe anemias of infancy* (pseudoleukemia infantum-Von Jaksch). In these cases, histologic examination reveals an excessive proliferation of myeloid tissue with predominant erythropoeisis. Because myeloid metaplasia of the spleen was also found in rachitic splenomegaly, Aschenheim, and Benjamin contended that Von Jaksch's anemia was due to this disease.*

The viewpoint of Naegeli and Masugi is broader. They believe that in addition to rickets, various influences, such as syphilis and alimentary disorders may cause severe anemia in infancy. The anemia acts as a stimulus to the blood-forming organs. This in turn produces excessive hematopoietic activity because of the great reactive power of the mesenchyme in infancy. In accordance with this opinion, Von Jaksch's anemia is not a distinct

* Sasuchin and Hayaski in their study of rickets found marked proliferation of the reticulum fibers and regarded the splenomegaly as a result of interstitial splenitis due to toxins.

morbid entity, the splenic enlargement being the result of compensatory hyperplasia of hematic cells.

Since Cooley's first report in 1927, many cases of severe, chronic, usually fatal anemia in infants have been collected. These cases of "Cooley's anemia," on the basis of clinical and hematologic characteristics, would be included in the collective term of Von Jaksch's anemia. They present, however, certain constant additional clinical features which seem to establish this group as a

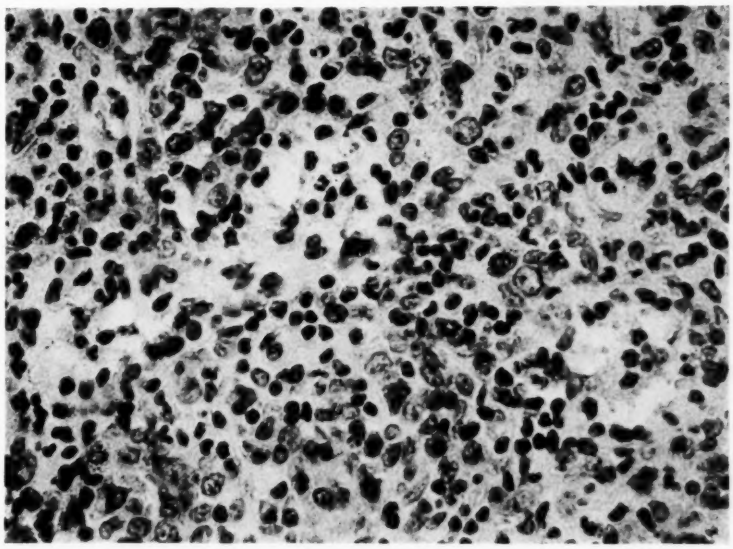


FIG. 18. COOLEY'S ANEMIA
Hyperplastic cellular pulp with but few hematic cells

definite morbid entity. These features are: (1) racial (Mediterranean) restriction, (2) familial occurrence, and (3) skeletal changes.

The histology of the enlarged spleen is not uniform (Whipple and Bradford). I have had the opportunity to study the spleen in eighteen cases. Dr. Martha Wollstein was kind enough to permit me to study her thirteen cases; two cases were offered to me by Dr. A. R. Kantrowitz; one by Dr. B. M. Vance. In the majority of these observations, the splenic pulp was very cellular,

the Billroth's cords were widened, and the sinuses were occasionally conspicuous. The Malpighian corpuscles were often small, sometimes strikingly reduced in number. A conspicuous increase in the number of reticulum cells was found in eight cases in which there was no thickening of the fibrillar reticulum (fig. 18). The cytoplasmic reticulum was also increased in six other cases and contained bizarre nuclei, but in these there was in addition a thickening of the reticulum fibers, beginning apparently about

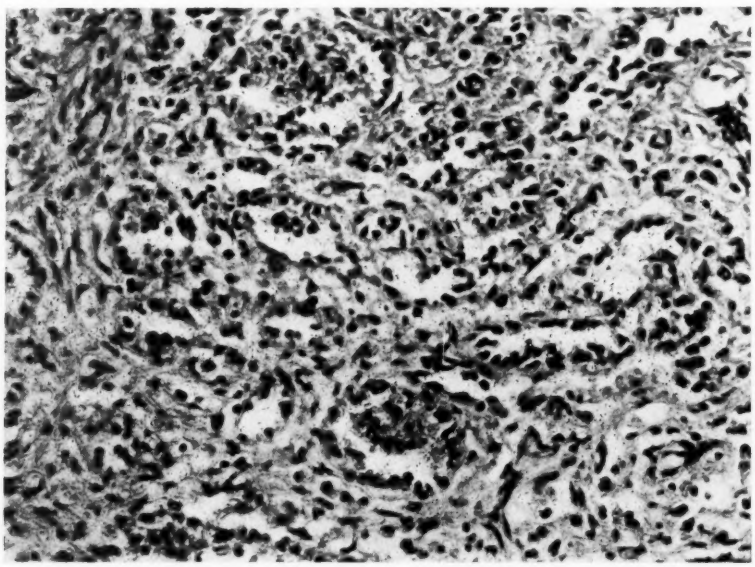


FIG. 19. COOLEY'S ANEMIA
Fibrosis of the intersinusoidal cords

the arterioles of the red pulp. In three of these, the thickening was so outstanding that the splenic pulp presented the appearance of fibroadenie (fig. 19). The increase of the fibrillar reticulum was most marked in the perifollicular zone of the red pulp (fig. 20). In these cases there was also peri-arterial fibrosis in the follicles. In seven cases only, were nucleated erythrocytes and nests of hemocytoblasts and myelocytes present in great numbers (fig. 21). The myelocytes, chiefly of the esosinophilic variety, and the

hemocytoblasts were arranged in nests, pointing to their local origin. The large "Gaucher-like" cells described by Whipple and Bradford were conspicuous in six cases (fig. 22). They were usually found about the small arterioles of the pulp, and did not stain with sudan III. Iron pigment was found in varying amounts, but never in excess. Whipple and Bradford described iron-free pigment within the sinus endothelium. I was unable to find it in my observations. Leukocytic and plasma cell infil-

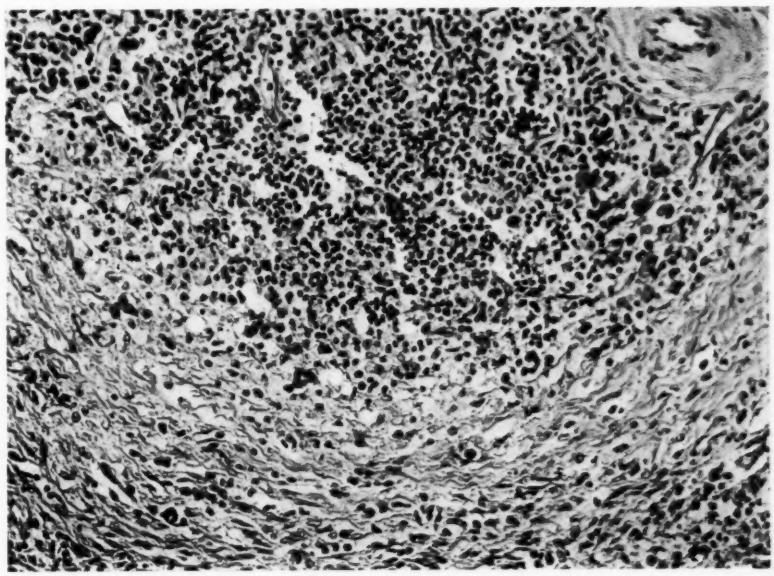


FIG. 20. COOLEY'S ANEMIA
Perifollicular fibrosis

tration of the pulp was striking in one case, even though necropsy did not disclose any terminal infection to account for this apparent inflammatory splenic alteration.

To summarize my findings, it may be stated that the splenomegaly in Cooley's anemia is caused chiefly by a conspicuous reticulum cell proliferation and in the further evolution of the disease by striking hyperplasia of the fibrillar reticulum. The nuclear changes of the reticulum cells observed in this late phase

indicate degeneration. One may consider this as an exhaustion stage of the over-stimulated cytoplasmic reticulum, followed by a terminal stage of fibrosis (fibro-adenie). As to the nature of the stimulus which causes the primary proliferation, the histologic analysis does not offer any suggestion. Since excessive blood cell formation was found in less than half of this series of cases, it seems that the reticulum cell hyperplasia is not caused solely by

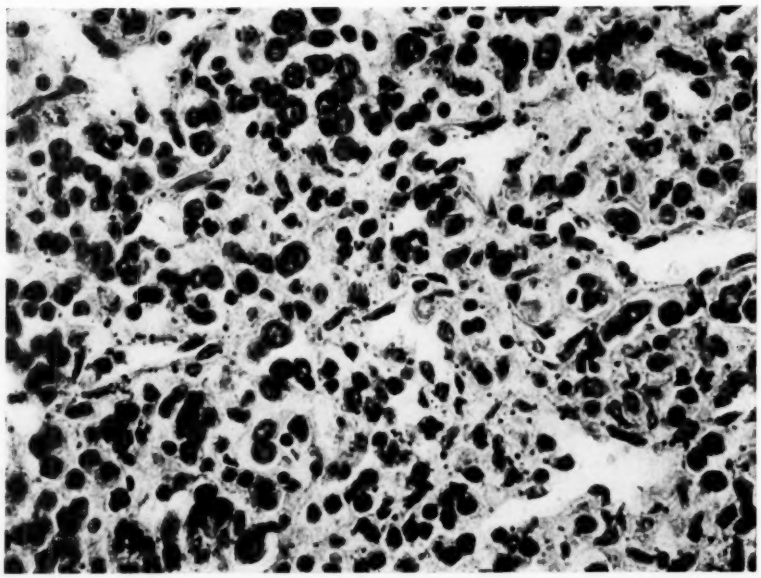


FIG. 21. COOLEY'S ANEMIA

Hyperplastic pulp with nests of immature hematic cells and erythroblasts

the stimulus which calls forth extra-medullary blood formation in the spleen.

Diffuse myeloid hyperplasia of the spleen and absence of malpighian corpuscles occurs in the so-called *erythroblastosis of the new-born* (figs. 23, 24). This disease is characterized by congenital icterus, splenomegaly, an enormous number of erythroblasts in the circulating blood, and occasional edema (Schridde's hydrops congenitus). The histologic appearance of the spleen resembles that found in an early period of embryonal life, and

may be regarded as a result of the arrested development caused by an alteration in the coördinate development of the various centers of the hematopoietic mesenchyme.

The most important subdivision in the group of splenomegalies due to hyperplasia is that which occurs in diseases of the hematopoietic system, conveniently called *hemoblastoses* (Orth). In myelosis there is almost always a striking, often enormous splenic

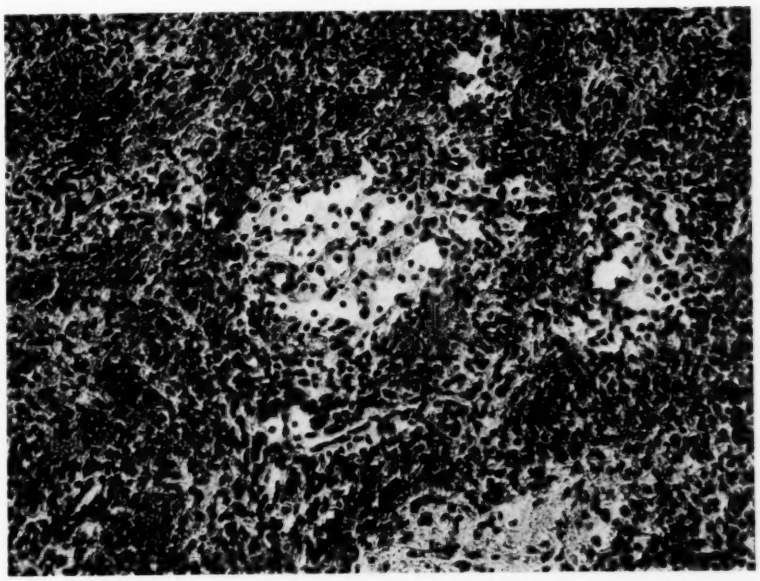


FIG. 22. COOLEY'S ANEMIA

Nests of large foam cells around pulp arteriole. (Courtesy of Dr. M. Wollstein, Baby's Hospital, New York, N. Y.)

enlargement, while in lymphadenosis the spleen is often only moderately enlarged. A detailed discussion of these conditions is omitted because it is adequately presented in text books of general pathology and hematology. I have discussed their special features in a previous paper (1934). Diagnostic difficulties for the clinician may arise in aleucemic cases, in which examination of the blood does not reveal changes. In such instances the diagnosis of "splenic anemia," or thrombocytopenic purpura may

be made, particularly if the hemorrhagic diathesis is striking. I have seen a number of cases in which splenectomy has been performed.

The so-called splenomata should also be mentioned in this group, even though they rarely exceed the size of a cherry and do not as a rule produce splenic enlargement. They have been re-

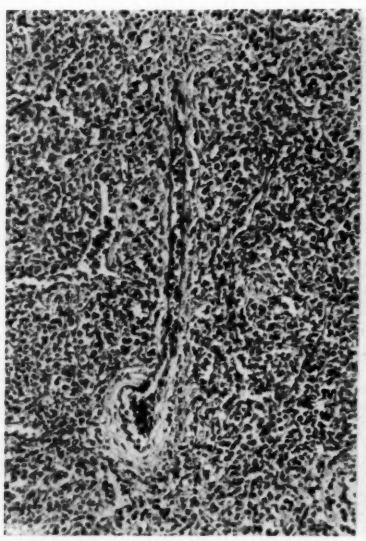


FIG. 23

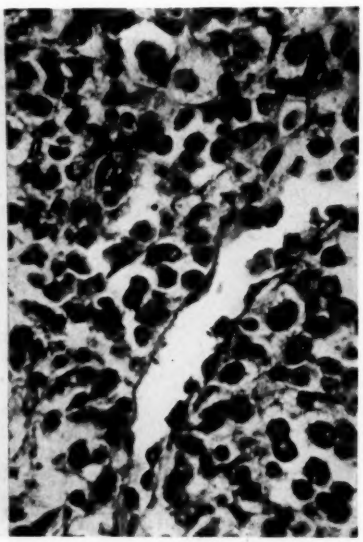


FIG. 24

FIG. 23. ERYTHROBLASTOSIS OF THE NEWBORN

Diffuse myeloid metaplasia of the red pulp. Note large arteriole without lymphatic follicle.

FIG. 24. ERYTHROBLASTOSIS OF THE NEWBORN

Numerous erythroblasts and other immature hematic cells

garded as nodular hyperplasias of the splenic tissue, but Lubarsch considers them as intra-splenic accessory spleens.

4. SPLENOMEGALY DUE TO NEOPLASMS

Primary neoplasms of the spleen are very rare (Lubarsch, 1927). Smith and Rusk, reviewing 104 cases in the literature, divide the malignant tumors into four groups: (1) endotheliomata (endothe-

lial sarcomata or angio-sarcomata); (2) lymphosarcomata; (3) round cell and fibrosarcomata; (4) miscellaneous group. The lymphosarcomata are most frequently encountered. They are often metastatic tumors, but can also originate within the lymphatic tissue of the spleen (fig. 25). Follicular lymphoblastoma produces a very conspicuous enlargement of the spleen. The cut surface is strikingly riddled with innumerable lymphatic nodules

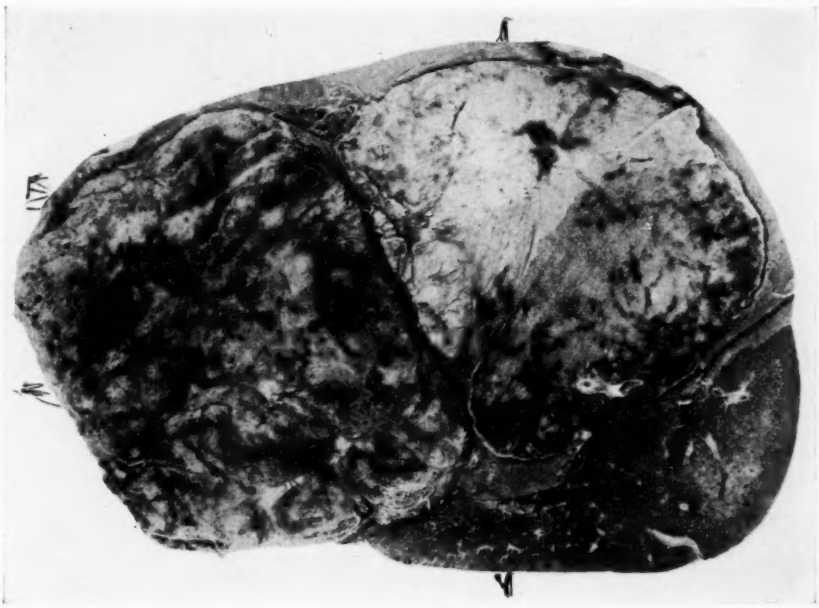


FIG. 25. PRIMARY LYMPHOSARCOMA OF THE SPLEEN
(Courtesy of Dr. M. Lederer, Jewish Hospital, Brooklyn, N. Y.)

(McNee, Ferrata). This form, however, is probably not a malignant neoplasm of the spleen, but an intermediate group between aleukemic lymphadenosis and lymphosarcoma (Baehr, Klemperer, and Rosenthal; Klemperer, 1934).

Non-malignant neoplasms of the spleen are even rarer than the malignant ones. Cavernous hemangiomata, lymphangiomata, and fibromata have been reported. The latter might be primarily angiomatous with secondary organization and obliteration of the vascular spaces analogous to the fibrous cavernomata of the liver.

Hemangioendotheliomata of the spleen may be part of a systematized neoplasia of the mesenchyme involving, in addition, the bone marrow and liver (Paine).

Metastatic tumors of the spleen are likewise rare, and seldom produce conspicuous splenomegaly. I have seen them chiefly in bronchus carcinoma. Pancreatic or renal neoplasms may invade the spleen by continuity.

FIG. 26. CYSTIC LYMPHANGIOMATA OF THE SPLEEN
(Courtesy of Dr. S. A. Goldberg, Presbyterian Hospital, Newark, N. J.)

5. SPLENOMEGALY DUE TO CYSTS

Fowler distinguishes pseudo-cysts and true cysts. The former are either traumatic or the result of focal degeneration of splenic tissue (infarct cysts). True cysts of the spleen may be classified as parasitic, non-parasitic and dermoid cysts (Andrals and Kamuris' case). Only the first two forms cause conspicuous splenic enlargement and might necessitate surgical intervention. Echinococcus cysts are the most numerous of the parasitic cysts. The true cysts are either of lymphangiectatic or of neoplastic origin representing cystic hemangiomata or lymphangiomata (fig. 26). They are either uni- or multi-locular.

6. SPLENOMEGALY DUE TO CIRCULATORY DISTURBANCE

Chronic disturbances in the splenic blood circulation play an important rôle in the development of certain well defined forms of splenomegaly.

Hyperemia of the spleen is either active or passive. Experimentally, the former can be produced by denervation (Henschen),

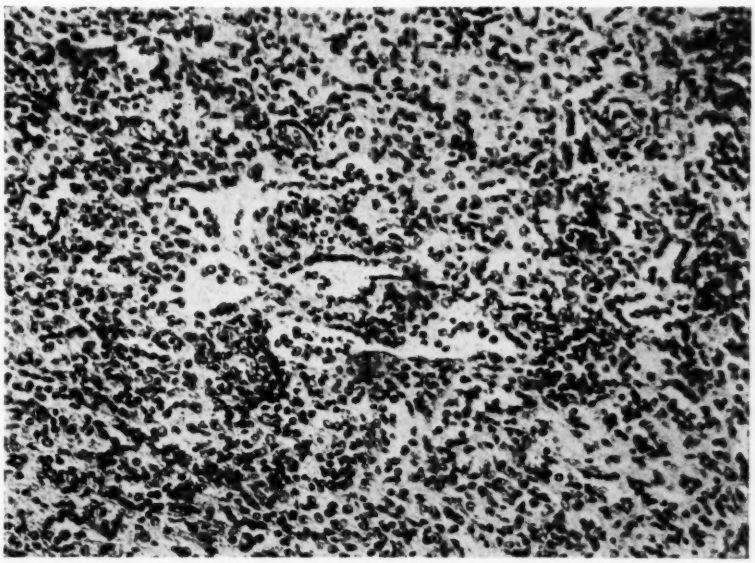


FIG. 27. CHRONIC PORTAL VEIN THROMBOSIS CAUSED BY PENETRATING DUODENAL ULCER

(Emergency splenectomy performed because of accidental injury to splenic vein during gastro-jejunostomy.) Early phase of splenomegaly. Widening of Billroth's cords due to reticulum cell (pale nuclei) proliferation. Dilatation of sinuses.

the latter by ligation of the splenic vein and subsequent division of the venous collaterals. Active hyperemia of the spleen is histologically characterized by engorgement of Billroth's cords and by the poorly filled sinuses; while passive hyperemia by an initial congestion of the sinuses with subsequent infiltration of the intersinusoidal cords, by erythrocytes. Observations in human pathology, indicate that the character of the changes occurring within

the spleen varies with the type of venous obstruction, i.e. whether the obstruction is due to central or portal causes. Acute cardiac stasis produces only moderate enlargement, and the spleen is very firm and dark red (cyanotic induration). After prolonged stasis due to right cardiac failure the spleen occasionally is considerably enlarged. It might, however, be smaller than normal (cyanotic atrophy). Microscopically, there is, at first, engorgement of the venous sinuses and of the pulp. In prolonged cases, the mal-

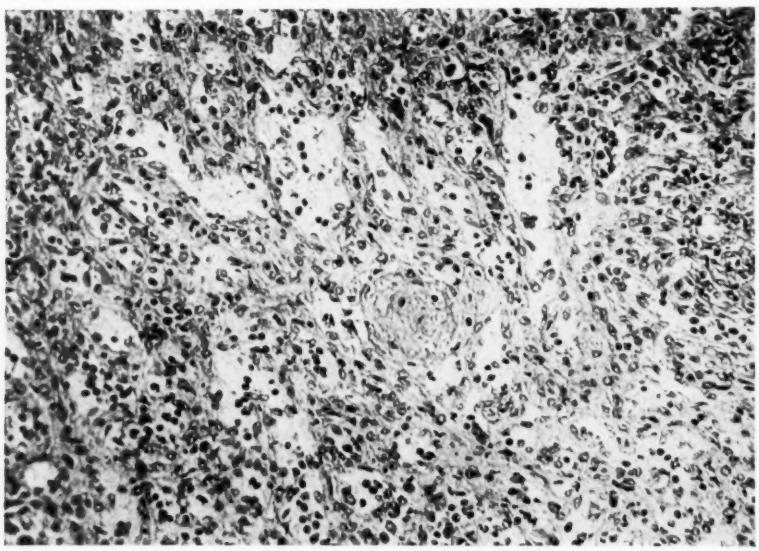
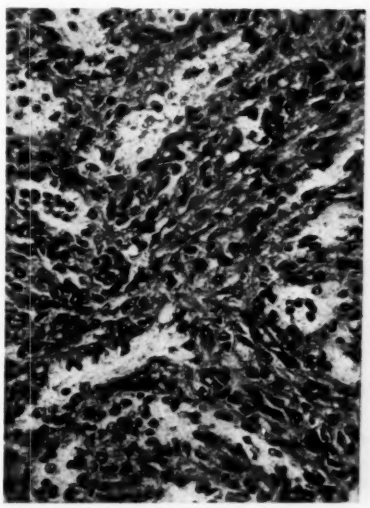


FIG. 28. EARLY SPLENIC VEIN THROMBOSIS

(Case of thrombocythemia. Death occurred 25 months after splenectomy.) Histologic appearance similar to that of figure 27.

pighian corpuscles might decrease in size, the cytoplasmic reticulum and the free white cells of the red pulp diminish in number, the reticulum fibers become slightly prominent and the capsule and trabeculae thickened. Venous stasis due to peripheral obstruction as it occurs in acute splenic or portal vein thrombosis at first causes a state of engorgement similar to that in acute general stasis. When prolonged, however, it leads very frequently, though not always (Wohlwill), to conspicuous splenic enlarge-

ment. This is striking in chronic thrombotic occlusion of the splenic or portal vein (thrombo-phlebitic splenomegaly, Bonne, Eppinger, Klemperer, Brugsch, Nobel and Wagner). Chronic occlusion limited to the splenic vein is less frequent than that of the portal vein. In 3500 necropsies, I observed three cases of the former and five of the latter (literature—Brugsch). The splenomegaly may develop soon after thrombosis has occurred. I have seen early organization of a thrombus in the splenic vein asso-



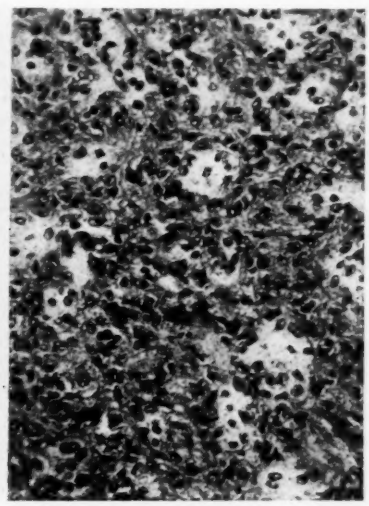


FIG. 29 FIG. 30

FIG. 29. HIGH POWER OF FIGURE 27
Conspicuous reticulum cell proliferation
FIG. 30. HIGH POWER OF FIGURE 28

ciated with a splenomegaly of 660 gms. Sclerosis of the portal or splenic vein might also be associated with splenomegaly. The enlarged organ has a rubbery feel, the capsule is thickened and often shows a marked perisplentitis. The cross-section is fleshy, salmon-pink, and frequently presents small circular hemorrhages and large or small yellowish-brown nodules (tobacco nodes of Gamna-Gandi). The latter two, however, also occur in other conditions (Oberling). The malpighian corpuscles are relatively reduced in number. Their size varies with the age of the patient.

Microscopically, in the early stages there is a widening and definite increase of sinuses together with a conspicuous diffuse hyperplasia of the cytoplasmic reticulum (figs. 27, 28, 29 and 30). Later a thickening of the fibrillar reticulum of the pulp (fibroadenie) (fig. 31), and of the follicles (fig. 32) is noted. The meshes of the pulp are narrow (fig. 35) and there are focal areas of hyperplasia of the cytoplasmic reticulum. The characteristic and outstanding feature is an apparent conspicuous increase in

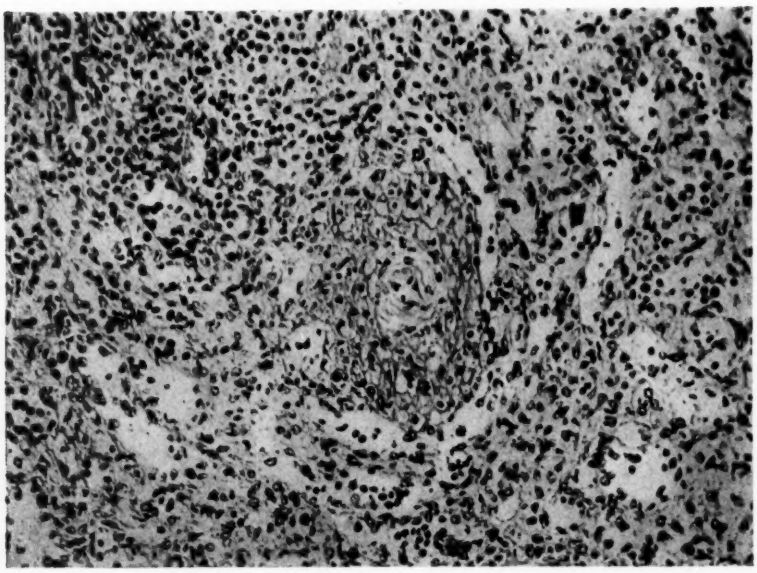


FIG. 31. EARLY SPLENIC VEIN THROMBOSIS
Early fibrosis around pulp artery

the number of the venous sinuses. The red pulp seems to be perforated by innumerable channels as if the Billroth's cords had been canalized (fig. 37). This condition has been designated as sinus hyperplasia (Dürr, Klemperer, Jäger). Hemorrhages are found about the trabecular arteries and at the periphery of the follicles (fig. 33) (peri-ellipsoid hemorrhages, McNee). The latter frequently become organized and leave characteristic nodular areas of fibrosis (McMichael) (fig. 34). The brown nodules are

the remnants of larger trabecular and peritrabecular hemorrhages. They contain yellow brown and green pigment granules and a similarly colored brushwood-like arrangement of fibers (fig. 44). On histo-chemical examination the pigment consists of iron and calcium phosphates. These fibers were regarded by Pinoy and Nanta as mycelia of aspergillus which had become encrusted with iron. Critical re-examination by other authors has shown that they are connective tissue and elastic fibers which have become

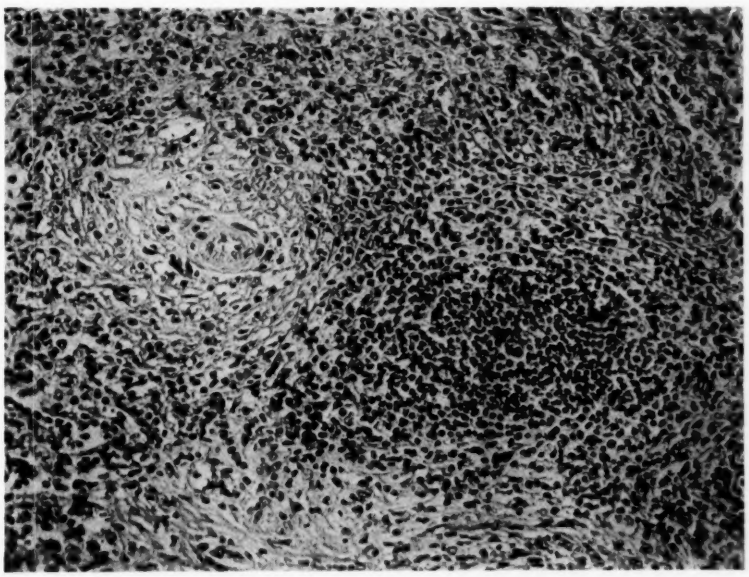


FIG. 32. EARLY SPLENIC VEIN THROMBOSIS
Early fibrosis around follicular artery—"fibroadenie"

impregnated with iron and calcium subsequent to the peri-arterial hemorrhage. A frequent additional histological feature is the presence within the sinuses of multi-nucleated giant cells of megakaryoctic type (fig. 36). These might be an indication of incomplete myeloid metaplasia of the splenic pulp, since definite myeloid metaplasia is found in rare instances of splenic or portal vein obstruction.

The outstanding differences in appearance of the spleen in

chronic central and peripheral stasis is explained by the fact that in the former the entire venous drainage of the spleen is under increased pressure, while in the latter, the collaterals are not compromised (Nishikawa, Hueck, Jäger). Chronic central stasis leads, according to Hueck (1929), to stagnation of blood within the chambers of the red pulp, thus only a minimal circulation is maintained through the direct arteriovenous channels (Hueck, 1928). The exclusion of the splenic pulp from active circulation

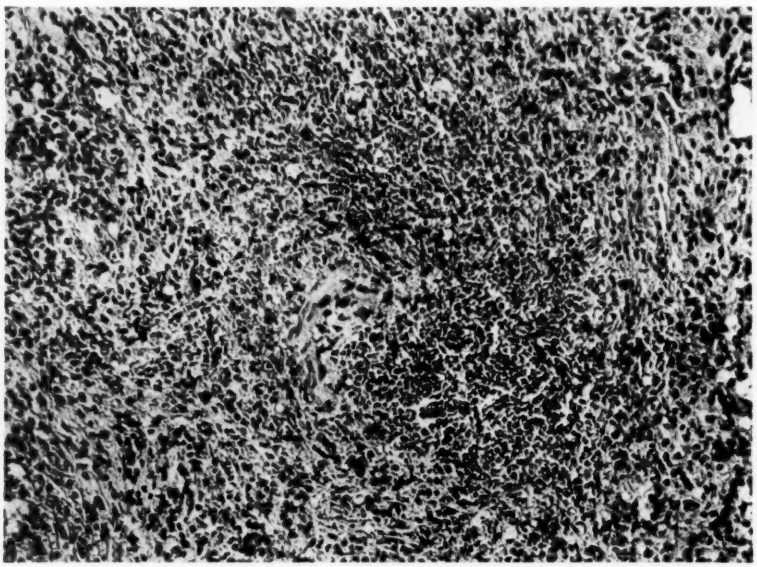


FIG. 33. CHRONIC PORTAL VEIN THROMBOSIS
Periarterial hemorrhage in vicinity of malpighian corpuscle

causes it gradually to atrophy. In peripheral stasis (acute splenic or portal vein thrombosis, ligation of the splenic vein) there is, at first, an engorgement of the spleen identical with that seen in acute central stasis. However, stagnation of blood and exclusion of the splenic pulp do not supervene since the collateral venous circulation is unaffected. The degree of stasis changes with the daily variations of the arterial circulation. The relative and intermittent stasis leads to hyperplasia of the pulp and not to its

atrophy. In a recent paper McMichael expressed the opinion that stasis alone is not responsible for conspicuous splenomegaly in instances of splenic or portal vein thrombosis. He believed that a co-existing hepatic disease, which might be recognized only microscopically, is the chief cause of striking enlargement, while stasis alone can only produce a moderate splenomegaly. The splenic enlargement in hepatic cirrhosis shall be discussed in a subsequent paragraph. However, it may be mentioned here,

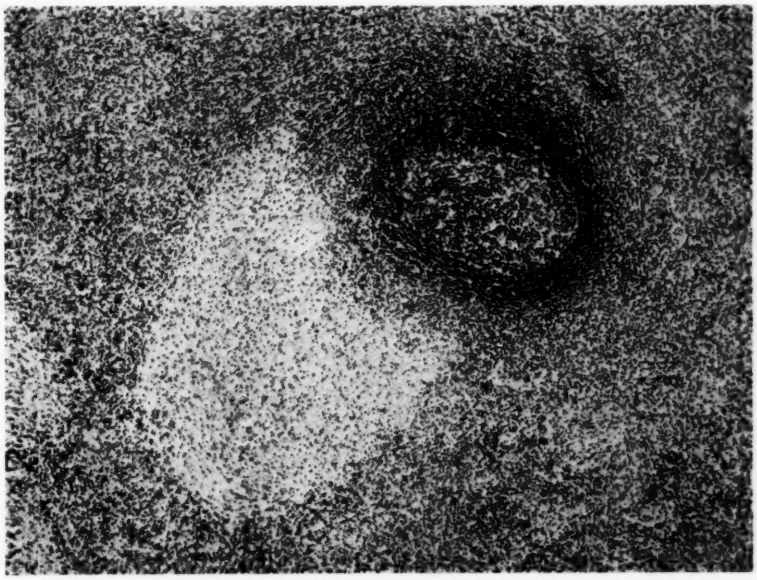


FIG. 34. CHRONIC PORTAL VEIN THROMBOSIS
Periarterial fibrosis in vicinity of malpighian corpuscle

that some factor in addition to the one of portal stasis must play a rôle in this disease. Nevertheless, in cases of isolated splenic vein occlusion it seems permissible to regard the often excessive splenomegaly as the result of chronic stasis alone. The same may be true of such cases of chronic portal vein occlusion in which the cause of the initial thrombosis can be traced to primary or secondary disease of the vascular wall or to alterations in the composition of the blood (Kaspar, Kratzeisen, Klemperer).

Furthermore, a histologic picture identical with that seen in thrombophlebitic splenomegaly in man was found in dogs in which chronic intermittent splenic stasis was produced (Jäger).

In the preceding paragraphs, the forms of splenomegaly have been divided into six groups and the essential anatomic features of each have been briefly presented. Most of the clinically recognized splenomegalies could thus be properly classified. There still

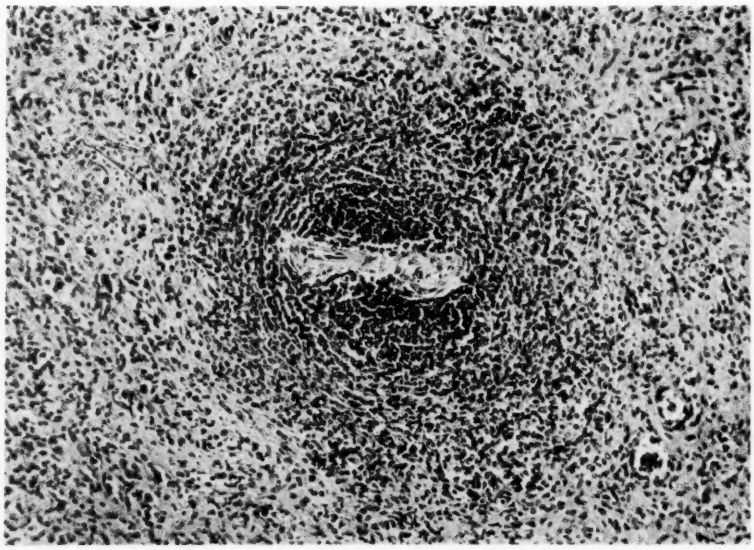


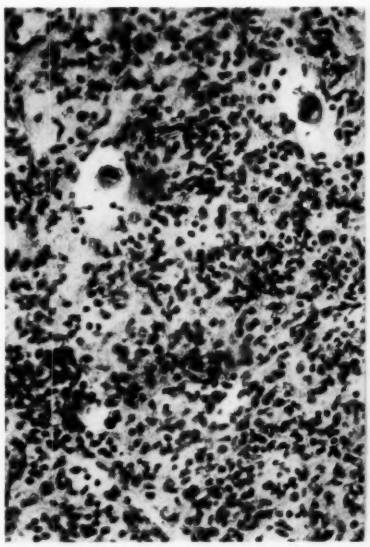
FIG. 35. CHRONIC PORTAL AND SPLENIC VEIN THROMBOSIS WITH CAVERNOMATOUS TRANSFORMATION OF THE HEPATO-DUODENAL LIGAMENT
(Case of thrombocythemia.) Advanced fibrosis of pulp

remain, for morphologic classification, some forms of splenomegalies which have thus far been grouped on a clinical basis only.

SPLENOMEGALY IN CIRRHOSIS

In liver cirrhosis, the spleen is frequently enlarged. The incidence of splenomegaly in liver cirrhosis varies from 72 per cent to ninety-two per cent (Oestreich; Klopstock; Bleichroeder). McCartney reports even 97 per cent. My own figures, based upon only fifty-six observations in the last seven years, shows enlarge-

ment in 79 per cent. If each form of this type was considered separately, splenic enlargement occurred in 77 per cent of the Laennec forms, in 66 per cent of the toxic cirrhosis, and in all of the biliary and indeterminate forms. Prominent splenomegaly, however, with splenic weights above 500 grams, was found only in Laennec cirrhosis and in the indeterminate forms.



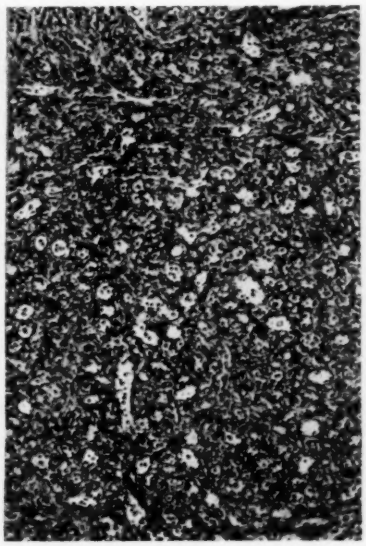


FIG. 36 FIG. 37

FIG. 36. CHRONIC PORTAL VEIN THROMBOSIS
Giant cells within sinuses

FIG. 37. OBLITERATION OF SPLENIC VEIN
(Splenomegaly for 20 years.) Sinus hyperplasia

It has been frequently stated that hypertrophic forms of liver cirrhosis are commonly associated with excessive splenic enlargement. If one uses Rössle's classification of hypertrophic liver cirrhosis, it appears that hypertrophic Laennec cirrhosis and fat cirrhosis, as well as biliary cirrhosis, do not generally present a striking splenic enlargement. In cases of chronic icterus of long duration caused by inflammation of the small intra-hepatic bile ducts, both hepatic and splenic enlargements are conspicuous.

These cases are frequently diagnosed clinically as Hanot's biliary cirrhosis, but anatomically they belong to the domain of cholangitis lenta. In exceptional cases, toxic cirrhosis may present an excessive splenomegaly, as in case number 4 of Stroebe. In one of my cases of subacute yellow atrophy, the spleen weighed 720 grams.

Spleens weighing 1000 grams or more are observed in "the hypertrophic angio-hematotoxic cirrhosis" of Roessle, a form which is most probably identical with Eppinger's "splenomegalic cirrhosis with and without icterus." Roessle believes that the cirrhogenic poison in these cases, primarily and simultaneously attacks the vascular system of the liver and spleen because of its special affinity for the reticulo-endothelial system. In addition, a hematotoxic factor is associated with this process. The excessive blood destruction is evidenced clinically, by a hemolytic type of icterus with severe anemia, and anatomically, by large amounts of blood pigment within the spleen, liver and other organs.

The macroscopic appearance of the spleen in hepatic cirrhosis is fairly characteristic. The enlarged organ is of rubbery consistency and the capsule is thickened. The color of the cut surface varies in individual cases from salmon-pink to dark red. The malpighian corpuscles are small, and the trabeculae are distinct. Occasionally circular hemorrhages within the pulp, peri-arterial hemorrhages and deposits of brown pigment within the trabeculae are present which are similar to those seen in thrombophlebitic splenomegalies (fig. 44). Histologically, there is thickening of the reticulum fibers of the pulp with narrowing of the meshes (fibro-adenie) and proliferation of the connective tissue about the arterial ramifications and trabeculae; the fibrosis of the latter radiates into the adjacent pulp (so-called "Trabekel Aufsplitterung"). Sinus hyperplasia occurs very frequently, but is not constant (fig. 38). The congestion of the sinuses and pulp has no significance because of the terminal cardiac failure. The malpighian corpuscles are reduced in number, their size depending upon the age of the patient. The pulp often appears cellular, due to the infiltration of Billroth's cords by polymorphonuclear leukocytes and plasma cells and the proliferation of fixed reticu-

lum cells and histiocytes associated with erythro- and siderophagocytosis (figs. 39, 40). The significant histologic characteristic is the association of the features of chronic peripheral stasis with those of chronic inflammation. The latter features are independent of incidental terminal infections because the spleens removed at operation show identical findings.

It has long been recognized that in cirrhosis, the chronic portal stasis is not the sole cause of splenic enlargement. The chief

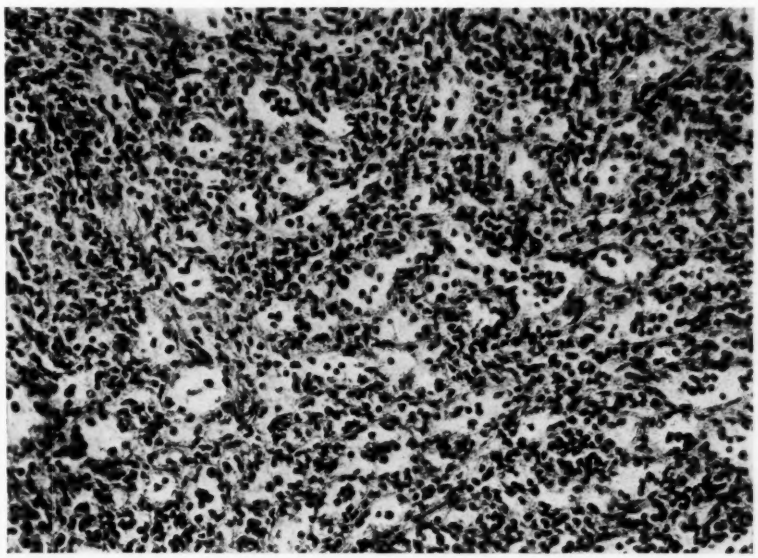


FIG. 38. HEPATIC CIRRHOSIS OF LAENNEC TYPE

(Fourteen year old female splenectomized for "Banti's Disease.") Increase and dilatation of sinuses. Widened and cellular Billroth's cords.

histological problem in this disease is the analysis of the nature of the reticulum thickening, the so-called fibro-adenie. According to Gauckler (1905) whose opinion is still shared by the French school, the spleen in cirrhosis is distinguished by a "hypertrophic pulp sclerosis." By this term, Gauckler designates a condition of the splenic parenchyma which begins with hyperplasia of the cytoplasmic reticulum and ends with fibrosis. The initial phase of hyperplasia is characterized histologically by an increase in the

number of clear nuclei in the red pulp. A mobilization of numerous macrophages occurs simultaneously. In later phases, differentiation of the proliferated cytoplasmic reticulum gives rise to collagenous fibers and a cellular connective tissue. In the terminal phases the formation of a compact acellular connective tissue, taking its origin from the trabeculae and the perivascular trabecular sheath, may cause contraction of the originally enlarged organ (sclerose atrophique). Gauckler's description of

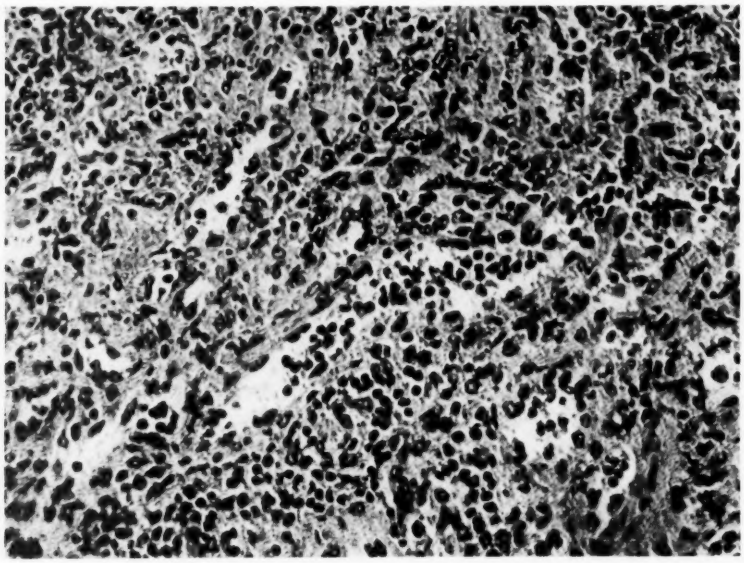


FIG. 39. TOXIC CIRRHOSIS OF LIVER

(Ten year old male splenectomized for "Splenic Anemia.") Conspicuous reticulum cell proliferation and inflammatory cell infiltration of Billroth's cords.

"sclerose hypertrophique pulpaire" coincides with Banti's definition of fibro-adenie, in which the reticulum fibers are conspicuously thickened and their meshes narrowed. This condition, according to Hueck's investigation, is the result of increase and thickening of the fibrillar reticulum, secondary to an alteration of the fibrillar material and the adjacent ground substance. Hueck believes this to be the result of a progressive gelation of the colloidal-sol phase of the ground substance. Gauckler sees

the reason for his "sclerose hypertrophique pulpaire" in the excessive demands upon the "plasmodial" (i.e., cytoplasmic) reticulum resulting from exaggerated erythrolysis. The latter is frequently the result of the primary liver disease, but may also be an independent primary process in the spleen. Hueck also regards the fibro-adenie, for which he prefers the term fibrosis or sclerosis, as a terminal stage of original hyperplasia of the cytoplasmic reticu-

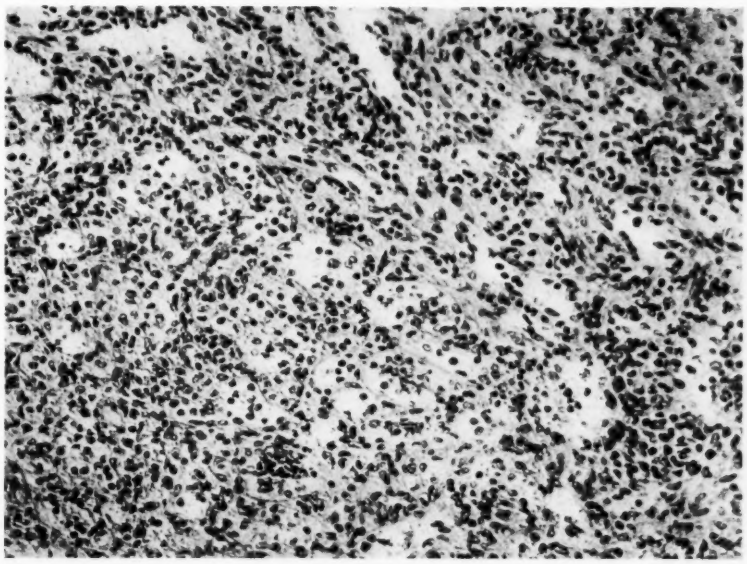


FIG. 40. COARSE NODULAR HEPATIC CIRRHOSIS

(Sixteen year old male splenectomized for "Banti's Disease.") Increase of sinuses. Widening of Billroth's cords due to reticulum cell proliferation and inflammatory cell infiltration.

lum but believes that this is produced not only by excessive erythrolysis, but may also result from over-stimulation of the reticulum by other factors. It is obvious, therefore, that fibro-adenie may follow any form of chronic hyperplasia of the cytoplasmic reticulum. (See the description of Cooley's anemia).

The factors which cause the irritation and proliferation of the splenic parenchyma (cytoplasmic reticulum) have been discussed in detail. Oestreich proposed a simple inflammatory factor. In-

deed a primary hepatitis may lead to cirrhosis and could obviously produce a concomitant inflammatory hyperplasia of the splenic reticulum. The splenic enlargement could thus be initiated by the inflammatory hyperplasia and become progressive because of the gradually developing portal stasis (Paltauf). This mechanism most probably prevails in cases of obstructive biliary cirrhosis (fig. 41) in which there exists not only an inflammatory stimulus, but also the influence of chronic cholemia, which alone,

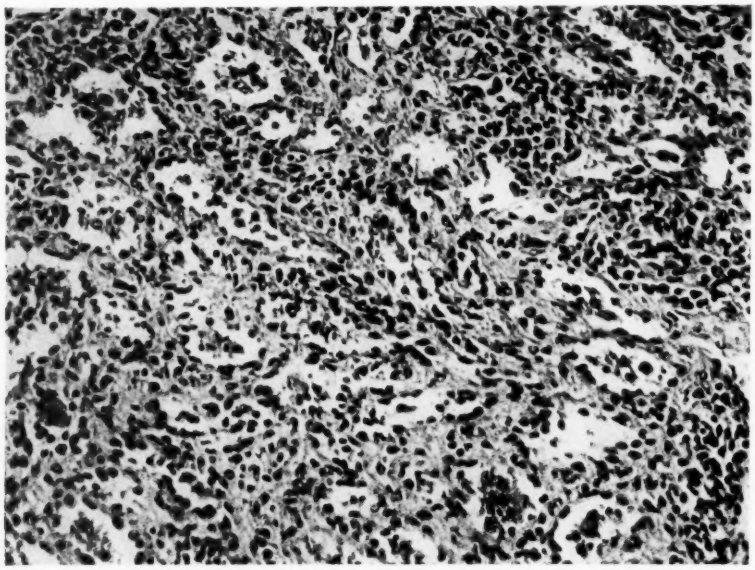


FIG. 41. OBSTRUCTIVE BILIARY CIRRHOSIS

(Weight of spleen at post mortem 500 grams.) Sinus hyperplasia and fibrosis of Billroth's cords.

can cause splenic swelling (Foa, 1907; Roessle, 1930). In these instances the splenic enlargement is secondary to hepatic alteration. On the other hand, inflammatory hyperplasia of the splenic reticulum might be due to the influence of an injurious agent which causes a simultaneous cirrhosis of the liver. Dietrich and Siegmund make reference to observations on "sepsis lenta," which presented a coarse nodular liver cirrhosis and a sclerotic splenic

tumor. In such cases the splenomegaly occurs concomitant with the hepatic alterations, and not subsequently; it is not dependent upon nor secondary to them. An analogous situation exists in the "angio-hematotoxic cirrhosis" of Roessle. In a number of such cases, the splenomegaly becomes clinically manifest at a period when there is no indication of a hepatic disorder.

The existence of cases of splenic enlargement in the precirrhotic stage of liver involvement was first stressed by Banti, as the initial phase of a hitherto undescribed morbid condition, the "splenomegaly with hepatic cirrhosis," which he believed to be a well-defined entity. It is commonly spoken of as "Banti's disease."

Banti not only outlined the clinical features but also emphasized definite anatomical alterations which, in his belief, characterized this disease as a distinct entity. At this point in the discussion, it must be made clear that subsequent writers on the subject did not always adhere to Banti's original criteria. From a clinical viewpoint, he stressed the fact that the disease occurred in young people (thirty-four of his patients were less than thirty-five years of age), and that it affected females almost twice as frequently as males. The complete absence of antecedent gastric symptoms, of diseases of the liver and biliary tract, of malaria, and of syphilis was emphasized. Alcoholism and dietary indiscretions were excluded as etiological factors. There were no indications that the disease was hereditary or familial.* Cases were not localized to any special sea level, being observed in the mountains as well as in the plains. The disease always ran a course of several years. The progress could be divided into three stages: (1) anemic phase with splenomegaly, asthenia and occasional hemorrhagic episodes, (2) transitional stage distinguished by gradually developing decrease in the urinary output, with urobilinuria, liver enlargement and a dirty brown discoloration of the skin replacing the simple anemia and occasional gastro-intestinal disturbances, and (3) ascitic stage with atrophy of the liver. In this stage there was no pronounced icterus, but the sclerae were sub-icteric. Hemor-

* Bastai recently described cases which were of a hereditary nature simulating Morbus Banti clinically but without characteristic splenic lesions.

rhages from the mucous membrane were observed. Death was usually the result of hemorrhages or of the autointoxication caused by the progressive hepatic atrophy. Apart from the conspicuous splenomegaly, the symptoms of the third period closely resembled those of atrophic cirrhosis. Banti was cognizant of the splenomegaly which occasionally occurred in atrophic cirrhosis.

Banti regarded the hematologic findings not as pathognomonic but as an aid in diagnosing the disease (Banti, 1910). To sum up, they consisted in a decrease of the total hemoglobin and of the erythrocytes (the latter not constant), absence of nucleated erythrocytes, frequent leukopenia with absolute or relative mononucleosis.

The pathologic alterations were present chiefly within the spleen and liver with almost constant findings in the splenic and portal veins. The spleen was markedly enlarged, the weight rarely less than 1000 grams. Histologically the splenic alteration was characterized by conspicuous thickening of the fibrillar reticulum in the malpighian corpuscles and red pulp. This lesion had previously been designated fibro-adenie by Banti (1883) because the characteristic reticular structure of the adenoid tissue was retained. Banti did not regard this lesion as the result of a genuine chronic inflammation because granulomatous foci were rarely encountered. When present, however, they were of a fibro-fascicular but no adenoid structure. It must be stressed that Banti himself maintained that the fibro-adenie of the red pulp existed in many diverse splenic disorders. "The fibro-adenic alterations in the follicle distinguish the Morbus Banti. Their absence rules out the disease." The changes originated either around the central artery or about its pre-follicular division. The progressive involvement of the follicles finally resulted in the transformation into sclerotic nodules. All the follicles did not show the same degree of fibrosis. Follicular alterations in various stages of development were encountered even in advanced cases.

According to Banti, the hepatic changes in the second and third phases could not be distinguished from those occurring in Laennec's cirrhosis. As early as the first period of the disease a chronic sclerosing endophlebitis of the splenic and portal veins was frequently observed.

In Banti's opinion these clinical and pathologic facts warranted the characterization of this condition as a new morbid entity. The negative results after a thorough search for bacteria and parasites led Banti to conclude that the disease was not caused by a known micro-organism. Nevertheless he regarded an infectious etiology as probable.*

Banti proposed the following hypothesis to explain the pathogenesis and progress of the disease. An unknown, probably infectious, agent reaches the spleen through the arterial pathway, localizing itself there and causing the fibro-adenie. At the same time toxins are elaborated within the spleen, which cause anemia, general debility and fibrosis. The last feature manifests itself not only by the development of the liver cirrhosis but also by the formation of sclerosing endophlebitis of the splenic and portal veins. The successful influence of splenomegaly upon the further progress of the disease is considered, by Banti, to substantiate his hypothesis, since the spleen acts as a nidus for the stimulus which produces the progression in the evolution of the disease. Many investigators accepted Banti's concept, but many others dissented from, and contested his views. Morbus Banti is, according to Eppinger, one of the most discussed problems of internal medicine. The literature on the subject is reviewed in the comprehensive presentations of Ziegler, Eppinger, and Mennet.

In a critique of Banti's disease, it is of paramount importance to determine whether the histologic changes in the spleen, as originally described, are encountered in other forms of splenometaly which by their etiology or known pathogenesis can be definitely distinguished from it. Inasmuch as Banti himself does not regard the reticulum changes in the red pulp as characteristic, the only question which remains for consideration is that of the possible occurrence of fibro-adenie of the follicles in other diseases. Banti stressed this as the characteristic alteration in his disease and illustrated it extensively in his latest article on the subject (1910). Attention should, however, be called to figure 18 of this

* The hypothesis of Nanta and his school that "Banti's Disease" is caused by fungus infection has been vigorously refuted.

article, which depicts a splenic follicle with distinct peri-arterial fibrosis in a case of alcoholic cirrhosis. A comparison of this with figure 8, designated as early fibro-adenie, shows a striking similarity. Mennet and Dürr found intra-follicular peri-arterial fibrosis in cases of hepatic cirrhosis, portal thrombosis and ulcerative endocarditis. I have described definite fibro-adenie of the malpighian corpuscles in thrombophlebitic splenomegaly (1928) and have made similar observations in other cases of obliteration of the splenic or portal veins (fig. 32) in hepatic cirrhosis (figs. 42, 43) and in Cooley's anemia. According to Hueck the localization of the fibro-adenie within the red pulp alone or within the follicles depends on the circulatory status within the latter, i.e. the presence of an internal vascular network, rather than on definite etiologic factors as Banti maintained. Since 68% of Banti's patients were under thirty-five years of age, and complete vascularization of the follicles occurs more frequently in young than in old people, the age incidence can account for the frequent follicular involvement. Hueck further contends that fibro-adenie is a morphologic feature of a terminal stage which might result from any form of chronic splenic hyperplasia. Because of this, the exclusively degenerative, toxic nature of the disease as maintained by Banti, cannot be accepted and since the alterations in the spleen as described by Banti are not specific for this disease alone, they cannot be regarded as diagnostic criteria.

Dürr studied ten original microscopic preparations from cases selected by Banti himself. In addition to fibro-adenie, he observed certain histologic changes which could possibly have been regarded as constant morphologic features of the disease. These consisted of marked sinus hyperplasia and indefinite demarcation of the trabeculae from the pulp. (Trabekelaufsplitterung). But identical features were observed in atrophic portal cirrhosis and other forms of splenomegaly. The sinus hyperplasia in particular is characteristic of chronic local venous stasis, as was mentioned above and the trabecular alteration is frequently observed in chronic inflammatory splenomegaly. Thus, even unbiased investigations of cases selected by Banti himself could not establish any specific histologic criteria. It is therefore evident,

that from the viewpoint of pathologic anatomy Morbus Banti cannot be regarded as a well defined morbid entity.

Albeit the histologic picture of the splenomegaly is not specific, the concept of Banti's disease as a distinct clinical entity could be justifiable if the symptomatology (i.e. chronic course, initial splenomegaly, anemia, asthenia and terminal atrophic liver cirrhosis) was rigidly adhered to. The clinical diagnosis, therefore, depends upon the characteristic evolution through all the

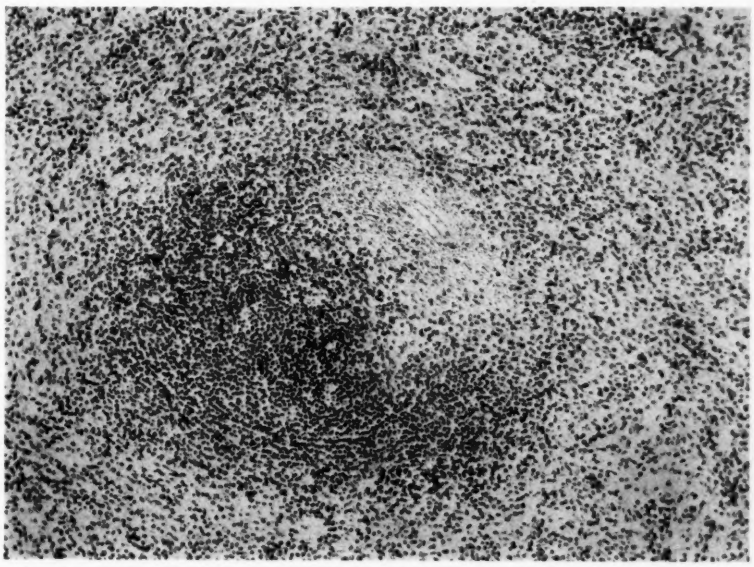


FIG. 42. COARSE NODULAR HEPATIC CIRRHOSIS IN WILSON'S DISEASE

(Twenty-one year old female splenectomized for "Splenic Anemia.") Fibroadenie of malpighian corpuscle.

three phases, and for this reason the disease can be justly diagnosed in the terminal phase only, after the preceding stages have actually been observed. Since the clinical picture is not characteristic and the histologic splenic lesions are not specific, a diagnosis in the early anemic period cannot be made with any degree of certainty. The alleged favorable results of splenectomy at this stage cannot be used as a basis for diagnosis of this disease, because there is no evidence to prove that the progress of the

disease was actually arrested. An improvement in the late cirrhotic stage also does not help to identify such cases because similar successful results after splenectomy have been reported in undoubted "primary" liver cirrhosis. The hematologic findings are certainly not pathognomonic, since anemia and leucopenia have been observed in many splenomegalic conditions as well as in atrophic cirrhosis (Nägeli). From the foregoing considerations

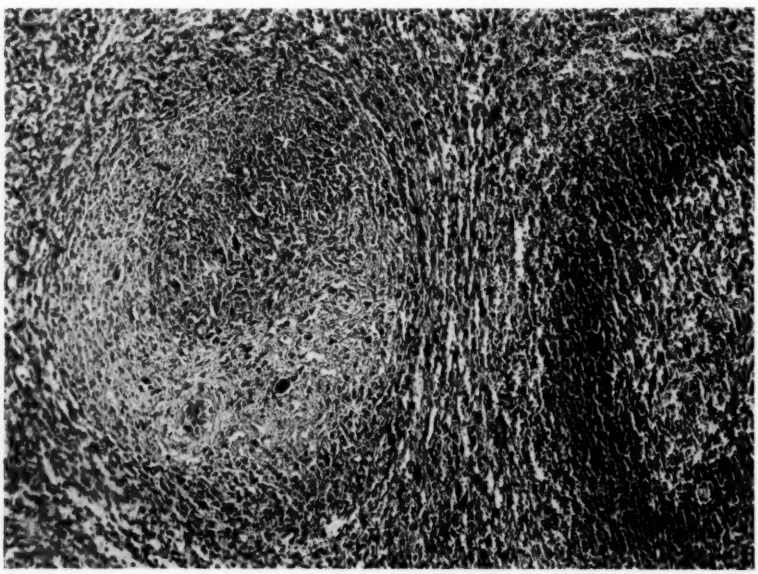


FIG. 43. COARSE NODULAR HEPATIC CIRRHOSIS

(Sixteen year old female splenectomized for "Banti's Disease.") Advanced fibroadenie of malpighian corpuscle.

it becomes apparent that the clinical symptoms likewise do not justify the recognition of Banti's disease as a distinct morbid entity. Because of the above clinical and pathologic considerations the diagnosis "Banti's disease" should be abandoned.

This standpoint has been accepted today by most pathologists and many leading clinicians (Lubarsch, Roessle, Warthin, Moschkowitz, Rolleston, McNee and others). On the other hand, a number of clinical authors, even though admitting the validity

of the arguments against Banti's disease as a disease entity, plead for the retention of the clinical concept of Banti's disease as a syndrome (Ziegler, 1914; Hirschfeld and Mühsam).

The complex of symptoms of the first stage has frequently been identified (Senator, Osler) with the clinical picture of "Splenic anemia."* Griesinger, Banti (1883.) The final picture of this latter form, with liver cirrhosis has been regarded as the second

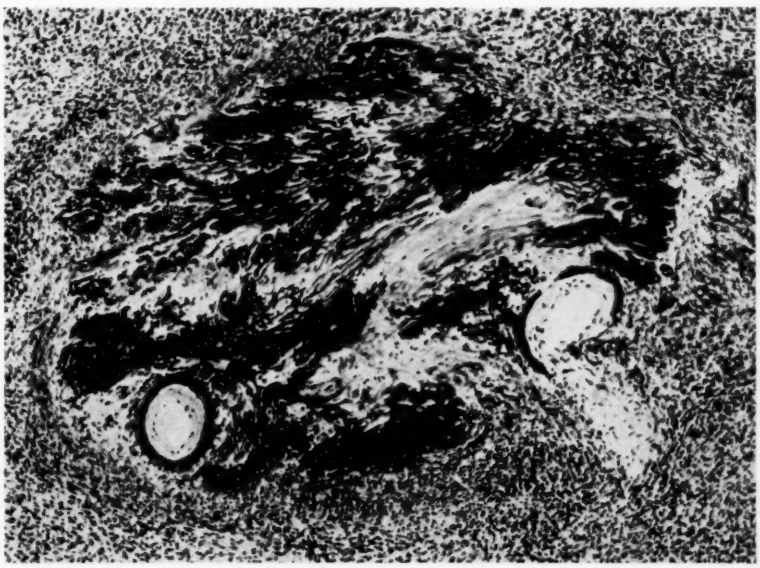


FIG. 44. HEPATIC CIRRHOSIS OF LAENNEC TYPE

(Thirty-three year old male splenectomized with a diagnosis of thrombophlebitis of the splenic vein.) Accumulation of pigment subsequent to hemorrhage—so-called tobacco node of Gandi-Gamna.

or Banti stage of splenic anemia. Accordingly, this stage might be reached in the progress of the disease but it is not a necessary consequence of the disease per se. (Senator.) Nageli and

* It may be mentioned, mainly for historical interest, that the very first case published by Groetschel as anemia splenica, was that of a ten month old infant and did not in any way comply with conditions which later were regarded as those of splenic anemia. It belonged to the group of splenomegalic anemias of infancy more than to the splenic anemia as now interpreted.

Sternberg (1905) have correctly stated that the term splenic anemia denotes mere coincidence of splenomegaly and anemia, but does not imply a well defined morbid entity. Because the term applies to a multitude of diseases of wholly diverse nature, its use certainly leads to confusion. Eppinger has summed up the many and diverse morbid conditions to which this diagnosis of convenience might be applied. The conditions often included in this group are, aleukemic myelosis, isolated splenic lymphgranulomatosis (Hodgkin's disease), primary neoplasm, tuberculosis, Gaucher's disease, thrombophlebitic and cirrhotic splenomegaly and finally even tropical diseases. From personal experience I could add cases of gumma of the spleen, amyloidosis, chronic sepsis (subacute bacterial endocarditis with splenomegaly (Libman)), echinococcus cyst, schistomiasis and even secondary carcinomatosis, where splenectomy was performed for "splenic anemia." In fact, any case with conspicuous splenomegaly and anemia might be placed in this meaningless group if not carefully studied. However, some cases remain undiagnosed despite the most painstaking clinical investigations, and in such instances the clinicians await information from the histological examination of the removed spleen. It must be admitted that these expectations are not always fulfilled, and that even the most careful histologic investigation at times, cannot solve the diagnostic problem. The reason for the anatomic uncertainty, in such cases, is the fact that the spleen presents the histologic picture of a sclerotic fibroadenic terminal stage which can follow heterogenous initial lesions, as has already been mentioned above, (fibro-adenie can originate from any primary hyperplasia). The aim is to correctly place the individual case on the basis of an exact histological analysis into one of the pathogenetic categories advocated above. When this will have been done in a sufficiently large number of cases, the diagnosis "splenic anemia or Morbus Banti" will automatically disappear from the clinical nomenclature.

In the anemias discussed above, the conspicuous enlargement of the spleen was the factor which brought the etiologic rôle of this organ to the fore. This view was supported by the apparent frequent immediate success of splenectomy. But the fact that

splenomegalic anemias are of such diverse natures speaks against the validity of such a concept. The histologic picture of the spleen in such cases certainly does not furnish any explanation for the origin of the severe anemia. The favorable result of splenectomy is probably due to a non-specific stimulation of the bone marrow. I have even seen a conspicuous improvement of anemia in a case of Hodgkin's disease after splenectomy.

The picture of *constitutional hemolytic anemia* (Minkowski-Widal) is more strictly outlined by definite clinical and hematologic criteria which need not be discussed in this paragraph. The belief in the etiological rôle of the spleen in this disease is apparently far better supported by the success of splenectomy, though the results are not always favorable (M. Freund). As a rule the spleen in these cases is considerably enlarged and markedly hyperemic. Microscopically, there is a striking engorgement of the reticular meshes of the red pulp, the venous sinuses being less distended (Eppinger, Guizetti, Freund, Thompson and others, *cf.* fig. 1 in Thompson's article). The enormous number of erythrocytes obscures other cells in the pulp, but in less hyperemic portions one finds a hyperplasia of the reticulum cells. The enlargement of the spleen is accordingly due to hyperemia and hyperplasia. Evidence of increased blood destruction is not constant, and erythrophagocytosis and siderosis, which are conspicuous in some cases, are absent in other instances. However, the constant bilirubinemia and the urobilinuria together with the ample siderosis in the Kupffer cells of the liver and in the epithelial cells of the convoluted tubules of the kidney, as found at necropsy leave no doubt that blood disintegration is increased in hemolytic anemia (Minkowski, Eppinger, Freund). There is some incompatibility between the success of splenectomy, pointing toward a primary blood destroying factor in the spleen, and the inconstancy of histologic findings evidencing this activity. Eppinger, in attempting to explain this contradiction, proposed the hypothesis which held that the disintegration of erythrocytes is only initiated in the spleen, and is completed within the liver. The most recent observations of Ceelen support this opinion. In two cases of congenital hemolytic jaundice, he found colorless

droplets and corpuscles lying within the splenic sinuses. These did not resemble hemosiderin particles, although their reaction for iron was positive. Ceelen regarded these structures as organic iron compounds, which possibly represented the first phase in the destruction of the red cell. However, the question as to whether this process is the result of a pathologically intensified function of the spleen or of an intrinsic disorder of the erythrocytes still is a moot question. The latter explanation is supported by the fact that the erythrocytes in hemolytic anemia present variations which lead one to suspect that they are more vulnerable than normal cells. On the other hand, the favorable effect of splenectomy suggests the primary influence of the spleen (hypersplenism). Eppinger adhered to the theory of the lienal etiology of hemolytic anemia, the characteristic engorgement of the splenic pulp lending morphologic support to his belief. He maintained that the engorgement of the reticulum creates unusual conditions favorable for increased blood destruction by the macrophages of the spleen. This excessive immigration of erythrocytes into the reticular meshes is accounted for by an alteration of circulation of blood. According to Weidenreich, whose opinion he accepts, the blood circulates inside the spleen within closed as well as open pathways, and alterations of the closed tracks could lead to a shunt of the current into the open pathways. Eppinger observed hyaline changes in the intima and rupture of the elastica within trabecular and central arteries, and believed that these alterations actually proved his physiological concept. However, identical vascular lesions are found in a great variety of diseases (Herxheimer, Matsuno) and therefore they cannot be of any specific significance in the causation of the characteristic splenic engorgement in hemolytic anemia. For this reason another explanation is necessary.

The engorgement of the reticulum meshes in the presence of relatively empty venous sinuses is the reverse of the picture found in venous congestion of the spleen, especially in the early phases. Here the venous sinuses are engorged first, and the reticulum meshes become filled later (Sokoloff). Gauckler and Menetrier, stressing this contrast, concluded that the histology of

the spleen in hemolytic anemia is distinguished by active (arterial) hyperemia. Arterial vaso-dilatation in the dog has been produced by denervation of the spleen (Henschen). It is significant that the histologic appearance of the spleen in these experiments closely resembles that of hemolytic jaundice (fig. 6 in Henschen's article). It therefore seems justifiable to maintain that the mechanism of the characteristic alteration in hemolytic anemia is arterial hyperemia caused by vasodilatation. Yet active hyperemia of the pulp per se produces no greater blood disintegration than does passive congestion. Hence, it in itself cannot be regarded as the primary cause of the increased erythrolysis in hemolytic jaundice. In hemolytic anemia, however, the erythrocytes may be primarily abnormal, and if such vulnerable elements come into such close contact with the reticulum cells they may undergo disintegration more readily than normal erythrocytes. Furthermore, the occurrence of the active hyperemia of the spleen can possibly be linked with the constitutional abnormality of the erythrocytes since active hyperemia of the red pulp is found after the intravenous injection of substances which damage the erythrocytes (toluidindiamin, Eppinger). Rich observed a very conspicuous engorgement of the perifollicular pulp in sickle-cell anemia. It is conceivable that the abnormal cells may initiate a reflex mechanism causing vaso-dilatation and resulting in active hyperemia of the splenic pulp. Such mechanism is suggested by experiments of Lauda.

According to my belief, the increased blood destruction in the spleen is dependent upon the abnormal circulation, but this abnormal circulation as evidenced by engorgement of the pulp is the result of the pathological condition of the erythrocytes and not of the splenic arteries. The fact that the evidences of their abnormality (i.e. decreased resistance, microcytosis) as a rule persist after splenectomy even though there is general improvement, can be more easily explained by such a concept than by the belief in damage to the erythrocytes by the perverted action of the spleen. In my opinion the success of splenectomy depends chiefly upon the removal of an organ which because of its unique circulatory properties is particularly capable of destroying abnormal

erythrocytes. It must be remembered that splenectomy may also favorably influence erythropoesis within the bone marrow.

The spleen in *pernicious anemia* may be moderately enlarged. The conspicuous blood destruction which occurs in this disease has attracted attention to the spleen. Interest in the microscopic structures was greatly increased after Eppinger's first reports on favorable results after splenectomy. Attempts were made to discover morphological explanations for this alleged key position of the spleen in the pathogenesis of pernicious anemia. Eppinger mentions engorgement as the most conspicuous feature of the spleen. This has also been observed by Benda, who examined the spleen in Kohan's cases. The erythrocytes accumulate chiefly within the reticular meshes, while the venous sinuses are almost empty. There is constant, though not excessive, presence of erythrophages and marked siderosis (Eppinger). The great quantity of pigment is present in the form of ferrous compounds. According to Eppinger the most important alterations are the vascular lesions, which are identical with those observed in hemolytic jaundice. Circulatory anomalies caused by these changes shift the blood into the open pathways of the pulp where the erythrocytes come in contact with the reticulum cells and are destroyed. The specificity of the vascular lesions has been refuted by all authors and their pathogenetic significance cannot be admitted. The amount of iron pigment in the spleen varies greatly (Lubarsch, Sternberg), the average amount being rather insignificant. Furtheremore, Lubarsch does not even admit a striking engorgement of the spleen. At any rate, it is not as conspicuous as that in hemolytic jaundice, and if present (Kohan-Benda) may be the result of irritation of the vaso-dilators, since the abnormal erythrocytes in pernicious anemia might act like those in hemolytic jaundice. Several authors have observed foci of extramedullary blood formation within the red pulp. Eppinger and Lubarsch describe conspicuous proliferation of the sinus endothelium in several cases.

Summarizing: the spleen in pernicious anemia does not present any lesion which is characteristic of the disease and there is no morphological evidence of exaggerated blood destruction

within the organ. This is in conformity with the present concept of the pathogenesis of pernicious anemia. The transient improvement after splenectomy must be accounted for by a stimulating influence upon the bone marrow. The exact nature of this stimulus is as yet undetermined.

THROMBOCYTOPENIC PURPURA

Although the spleen is not frequently enlarged in thrombocytopenic purpura, it has been regarded as a most important factor in the pathogenesis of this disorder, and the success of splenectomy seems to support this belief. According to Frank, the thrombopenia is primarily due to a faulty formation of the platelets within the bone marrow; according to Kaznelson it is due to their exaggerated destruction within the spleen (thrombolytic purpura). The possibility of capillary lesions caused by splenic products has also been taken into consideration by several authors. For these reasons the presence of histologic changes within the spleen could reasonably be expected. Kaznelson observed an accumulation of platelets, many of which were engulfed by macrophages, within the spleen pulp. These observations were confirmed by few authors only. The great cellularity of the spleen renders a recognition of platelets exceedingly difficult in tissue sections. In searching for phagocytosed platelets in several of these cases, I have seen only once macrophages containing platelets, and this was in a perfused spleen. In fresh spreads from the spleen, platelets are found more readily, but there does not seem to be an absolute increase in number as compared with control cases. The histologic examination of the enlarged spleen shows a widening of the Billroth's cords by reticulum cell hyperplasia. Occasionally myelocytes and sporadic megakaryocytes are encountered. In a few cases I was impressed by the conspicuous wide marginal zone of the malpighian corpuscles, but I am reluctant to regard this observation as a characteristic feature because similar findings are present in the spleens of healthy men and animals.

Summarizing: the histologic appearance of the spleen does not present either characteristic alterations or lesions which could account for the pathogenesis of the disease.

Within recent years I have studied the spleen in three cases in which the diagnosis of thrombopenic purpura had been made by competent clinicians.

In the first observation (Koster) the spleen was very large (600 grams in a twelve year old girl). Splenectomy was performed and a striking myeloid metaplasia which was suggestive of myelosis was found. The patient did splendidly after operation but three months later she had a recurrence of her symptoms and died shortly after. Necropsy revealed an aleukemic myelosis with myeloid infiltrations in all the organs.

In a second case likewise the spleen showed a diffuse infiltration with immature blood cells. In the further course, a leukemic blood picture developed and the glands became palpable. The child died and no necropsy was performed.

In a third case, a girl of four years, there was a large spleen, with multiple infarcts. The small arteries of the spleen contained numerous microscopic thrombi. The child died shortly after splenectomy. Necropsy revealed numerous thrombi within the arteries of all the organs. These three observations deserve short mention because they illustrate the fact that the picture of thrombopenic purpura can be produced by a systemic disease. In such instances the histologic examination of the spleen may reveal distinct lesions in contrast to the non-characteristic appearance in the majority of cases.

ERYTHREMIA (POLYCYTHEMIA VERA, OSLER-VAQUEZ)

In one group of these cases (Vaquez) splenomegaly is a characteristic symptom of the disease. Several authors regard the splenic lesion as causative. The development of the erythremia is explained either by increased erythropoiesis in the bone marrow or by a diminished destruction in the spleen. The occasional observation of isolated splenic tuberculosis (Rendu-Widal, Schalck, Sachs, Lit) associated with erythremia is regarded as supporting the latter viewpoint. Such lesions, however, are by no means consistently encountered and might possibly be incidental findings, since splenic tuberculosis is not, as a rule, accompanied by erythremia. Sachs stresses the fact that the splenic

tuberculosis in her case and other cases of the literature was more recent than the polycythemia, which had existed for at least five years and suggests that the erythremia might cause hyperactivity of the spleen, a condition favorable for the localization of the tuberculous process.

In other instances thrombosis of the splenic or portal veins was found (Lommel, Oppenheimer, Lit). Because cardiac stasis frequently results in secondary polycythemia, it was suggested that the local stasis within the portal circulation might be responsible for the development of polycythemia (Lommel). However, it seems more plausible that the increased coagulability of the blood in erythremia is the primary cause of the portal thrombosis. The belief is supported by the fact that in erythremia thrombosis occurs in many parts of the vascular tree. Moreover, polycythemia is not a frequent, and certainly not a constant phenomenon of portal stasis. Since these alterations are then considered as only incidental or secondary phenomena, it must be admitted that the macroscopic examination of the spleen in erythremia is not specific. The hyperemia is conspicuous but is present in all organs. Infarcts are frequently encountered. Histologically a striking engorgement of the sinuses and a conspicuous cellularity of the red pulp with but few malpighian corpuscles is seen (Delannoy). In his observations hyperplasia of the reticulum cells was noted. In one of my cases the red pulp consisted almost exclusively of large cells with abundant cytoplasm and clear nuclei. These were joined with one another by cytoplasmic processes forming a close meshwork and only a few red and white blood cells and a sporadic free histiocyte were found within the spaces. Neither free histiocytes nor the fixed reticulum cells showed any evidence of erythro- or siderophagocytosis. In two other cases the reticulum cell hyperplasia was not conspicuous. The paucity of iron pigment has been frequently stressed in the literature (Westenhoeffer). Eppinger attaching much importance to this feature, regards it as evidence of decreased blood destruction. However, the constant presence of abundant red bone marrow within the long bones speaks far more in favor of hyperactive erythropoiesis as the primary cause of

erythremia. It has not yet been conclusively shown that the spleen is actively engaged in the destruction of normal erythrocytes (Lauda) even though its digestive action upon abnormal erythrocytes is well supported by pathologic and experimental facts. The absence of blood pigment in the spleen in erythremia therefore must not be regarded as conclusive evidence of hypofunction of the spleen. It rather indicates that the excess erythrocytes formed within the bone marrow have a normal resistance and that the body has adjusted itself to their surplus. Erythremia has been frequently compared with leukemia, the hyperplasia of the erythroblastic elements being analogous to that of the leukoblastic mesenchymal tissue. The observation of proliferation of the cytoplasmic reticulum, as described above, could be regarded as a participation of the undifferentiated splenic mesenchyme in this hyperplastic process. The splenomegaly in erythremia might, therefore, be classified with those of hemoblastosis in the hyperplastic group. Even though this finding is not constant, the conclusion is not incompatible with this hypothesis, because the spleen is likewise not always involved in the hyperplasia of lymphatic leukemia, an obvious form of hemoblastosis. The occasional observation of myeloid foci within the spleen in erythremia (Hirschfeld) shows that the hyperplastic maternal reticulum might metamorphose into more mature cells. These instances are of great interest because of the transition of cases of erythremia into myloid leukemia (Parkes Weber, lit., Minot and Buckmann, Cahen).

SUMMARY AND CONCLUSIONS

Histologic investigation permits of a comprehensive classification of the various forms of splenomegaly based upon general pathologic principles.

The groups and their subdivisions are represented in the following table:

1. Inflammatory Splenomegaly
   - A. Chronic non-specific infections.
     Prototype: subacute bacterial endocarditis.
   - B. Chronic protozoal infections.
     Malaria, Kala-Azar.

C. Parasitic infections.
Schistosomiasis.
D. Tuberculosis (rare).
E. Syphili .
(1) Congenital.
(2) Acquired (rare).
F. Hodgkin's disease.

2. Infiltrative Splenomegaly.
A. Involvement of cytoplasmic reticulum.
Gaucher's, Niemann-Pick's disease and occasionally in diabetic lipemia.
B. Involvement of fibrillar reticulum.
Amyloidosis.

3. Hyperplastic Splenomegaly.
A. Compensatory or constitutional hematopoiesis in anemias.
Pseudo-leukemia infantum, erythroblastosis of the newborn.
B. Primary hematopoiesis.
Hemoblastosis, (Myelosis and lymphadenosis).
Polycythemia.
C. Idiopathic hyperplasia of reticulum cells and its derivatives.
Cooley's anemia (often associated with compensatory hematopoiesis).
Reticulosis, lymphatic hyperplasia in Grave's disease.
Constitutional splenic hyperplasia (Muehlmann).
Thrombocytopenic purpura.

4. Neoplasms.

5. Cysts.

6. Chronic Disturbances of Blood Circulation.
A. Active hyperemia, associated with secondary hyperplasia.
Hemolytic icterus.
B. Passive hyperemia, associated with secondary hyperplasia.
Obstruction of portal or splenic vein.
C. Passive hyperemia, associated with primary hyperplasia and chronic inflammation.
Hepatic cirrhosis.

The histologic characteristics of the individual groups are sufficiently specific to permit at least of a morphologic classification of such cases which clinically have been unclassifiable.

The third group, that of hyperplastic splenomegaly, is the least well defined, since various factors, which can be identified histologically as inflammation, infiltrations or circulatory disturbances can stimulate the proliferation of the cytoplasmic

reticulum and of its derivatives. In those instances, in which the cause of the hyperplasia can be ascertained from associated histologic features, the classification is made according to the pathogenesis. Hyperplasia of undifferentiated reticulum cells, however, can occur without displaying any features suggestive of any of the above causes. In such instances one has to be content with the mere descriptive diagnosis of iodiopathic hyperplastic splenomegaly. The splenomegaly in hemoblastosis has been classified as hyperplastic since the excessive blood cell formation in this morbid condition is regarded as an evidence of a hyperplastic form of hematopoiesis.

A critical histologic investigation of the spleen in cases of "Banti's disease" and "splenic anemia" reveals that specific and constant histologic criteria characteristic of these diseases do not exist. However, it becomes evident after histologic investigation that the individual cases can be grouped with one of the pathogenetic divisions. Most frequently these cases belong to the sixth group of splenomegaly, those due to chronic disturbances of the circulation, which are secondary to splenic or portal vein obstruction or hepatic cirrhosis.

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THE BLEEDING TIME*

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The hemostatic function of the body may be disturbed in various ways. Although not all factors sharing in this function are known and its exact mechanism is not well understood, its degree of efficiency or impairment may be approximately tested by a number of methods. Among these methods is the bleeding time. This discussion is limited to whatever new facts or conception seem to have been added to this topic in the last ten years.

The bleeding time may be defined as the time taken by a cut in the skin, of approximately uniform depth and length, to stop bleeding. The test was first described by Duke,[1] who also pointed out much of its significance and its relationship to the blood platelets. It is essential to think of the test as primarily one of the hemostatic power of the skin, and not of the individual as a whole. In patients with hemophilia, showing abundant evidence of bleeding in the muscles, joints and other organs, the bleeding time is usually normal, a paradoxical fact to those accustomed to it as an expression of the hemostatic function of the body in general. Given identical conditions, the skin will often behave differently from other tissues with respect to hemostasis. For example, in patients undergoing splenectomy for thrombopenic purpura, most of the bleeding during the operation comes from the lips of the skin incision and very little from the deeper tissues or the viscera. For these reasons, it may be preferable to refer to the test as the skin bleeding time.†

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† In subject indices this topic should be listed under any or all of the following headings: blood, hemorrhage, hemostasis, bleeding time; it should not be listed under coagulation of the blood.

METHOD

One of the aims in any technic is to produce a cut of approximately uniform depth and length. For this purpose automatic lancets can be used, or in some hands, a sharp knife is all that is needed. The device illustrated in figure 1 was built from a twelve blade scarificator from which all blades but one were removed, and is especially suited for use in experimental work. The depth of the cut in the skin can be regulated by a screw (E), which adjusts the distance that the knife blade (A) will project outside the instrument. By means of the spring (C) the blade is placed into position in the interior of the instrument; the apparatus is then laid against the portion of the body selected and the spring is released by pressing a button (D). The blade comes out, sweeps over a half circle into the skin so rapidly that no pain is felt. The outcoming blood is

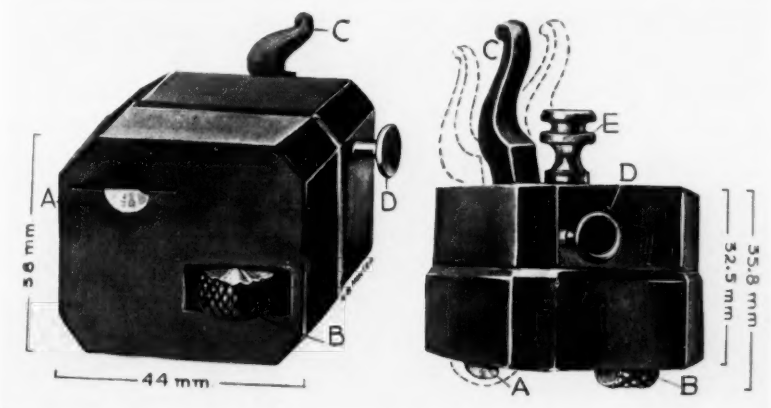

FIG. 1. AN AUTOMATIC DEVICE TO PRODUCE A CUT OF UNIFORM DIMENSIONS AND INFLICT A GRADED AMOUNT OF TRAUMA, TO TEST THE BLEEDING TIME AND PETECHIAL REACTION OF THE SKIN

taken up by blotting the wound lightly at 15 or 30 second intervals with absorbent paper. The instrument also carries a device for inflicting a graded amount of trauma to the skin without breaking its surface. This is accomplished by allowing a piece of metal (B) with a grated surface to sweep against the skin. The metal piece is operated by the same spring (C) and release button (D) that operate the knife blade. The screw (E) regulates the amount of trauma by adjusting the distance that the metal piece will project outside the instrument. By this means the surface of the skin over an area 10 x 8 mm. is scraped. Depending on the number of petechiae counted in that area five minutes after the trauma is applied, the result is recorded as 0, 1, 2, 3, et cetera. In man the dorsal surface of the forearm near the elbow was chosen for the tests,

prominent veins avoided, and the depth of the cut adjusted between 2 and 3 mm. In dogs the skin of the abdomen and thorax was used and cut to the same depth. The weight of the instrument is 206 grams, enough to keep it in close apposition to the skin while being operated. Both tests may be run simultaneously; they may be run separately by removing either the grated metal piece or the knife blade.

Ivy, Shapiro, and Melnick[2] have modified the test as described by Duke by applying a pressure of 40 mm. of mercury around the arm and taking the bleeding time in the forearm below. They believe this makes it possible to demonstrate the presence of a tendency to bleeding in jaundice and other conditions when other methods have yielded negative results. In normal individuals the duration of the bleeding time was slightly longer and there was a greater volume output of blood than when no pressure was applied. In patients with jaundice, however, application of the pressure often yielded a marked prolongation when the bleeding time as done by Duke's method showed little or no deviation from normal.

Instead of blotting the blood emerged from the cut, Roskam[3] washed it away by directing a fine jet of water at an even pressure and temperature at the wound. By this method he has been able to test the effect on hemostasis of various substances and physical changes.

VARIATIONS OF THE BLEEDING TIME

The bleeding time may be normal (60 to 180 seconds), slightly prolonged (180 to 300 seconds), long (300 seconds or more), or short (less than 60 seconds). Normal individuals show very little variation in the bleeding time taken at different times of the day even when the individual is fasting. Wide variations, however, may be found in those with hemorrhagic disease. In most types of catarrhal jaundice and in that accompanying cholelithiasis the bleeding time is prolonged.[11] There is no alteration found in hemolytic jaundice nor in the various types of cirrhosis of the liver with or without splenomegaly. In congestion of the liver due to cardiac, hypertensive or renal disease, and in Vaquez disease, there is nearly always a prolongation of the bleeding time,

sometimes very marked. In patients undergoing anti-syphilitic treatment with arsenical preparations, the bleeding time may be used as an index of the tolerance of the individual to the drugs; a prolongation is often one of the first signs of intolerance to the drug.[11] Besides these quantitative variations in the duration of

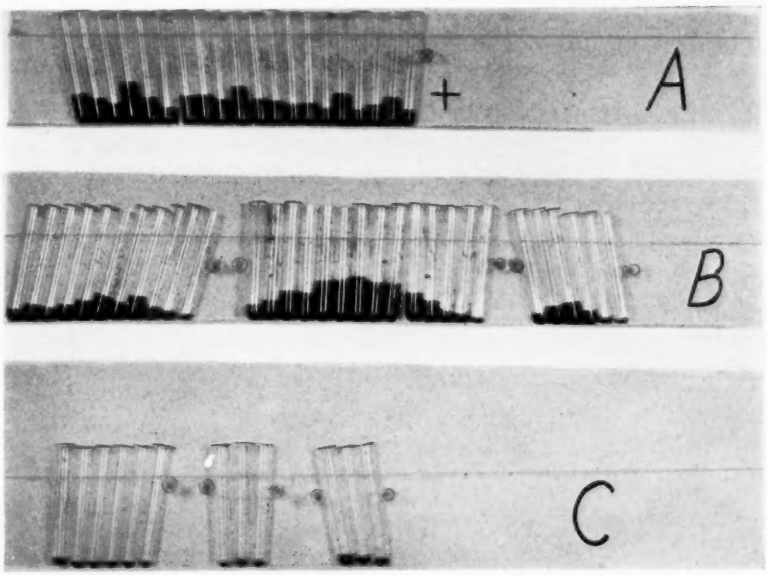

FIG. 2. MEASUREMENT OF THE OUTPUT OF BLOOD AT 15″ INTERVALS FROM A CUT IN THE EAR VEIN OF DOGS

Each tube (capacity 5 cc.) holds the blood collected during 15 seconds. (A) Prolonged bleeding time in dog with acute purpura. Increased in output and duration and arhythmic in delivery. Only the first five minutes are represented. (B) Three examples taken from normal dogs. (C) Three examples of short bleeding times from dogs, 3 to 8 days after recovery from acute purpura. Decreased rate and volume of output.

the bleeding time, there may also be variations in the volume output of blood from the skin wound (fig. 2). During the acute stage of purpura (human and experimental) the volume output of blood from a skin wound per second may be doubled, whereas during the period of recovery it may be cut down to insignificant proportions. When the output of blood from a cut is high, the

bleeding time is nearly always prolonged. There is, otherwise, very little correlation between the bleeding time and the volume output of blood per second. In humans the approximate amount of blood lost from the ear lobe every 30 seconds was calculated by measuring the area occupied by the blood drops in the absorbent

TABLE 1

RESULTS OBTAINED IN A FEMALE WITH THROMBOPENIC PURPURA

BLEEDING TIME	VOLUME OUTPUT OF BLOOD	BLEEDING TIME	VOLUME OUTPUT OF BLOOD
seconds	*cu. mm. per second*	*seconds*	*cu. mm. per second*
510	5.3	930	4.5
540	0.7	900	2.2
510	1.0	900	1.1
90	0.3	900	1.6
180	0.4	720	13.0
300	0.4	900	11.2
390	0.8	270	4.5
300	1.6	360	2.0
900	2.7	270	3.1
990	5.2	390	5.6
990	6.1	720	2.0

TABLE 2

READINGS FROM SIX NORMAL MEN

BLEEDING TIME	VOLUME OUTPUT OF BLOOD	BLEEDING TIME	VOLUME OUTPUT OF BLOOD
seconds	*cu. mm. per second*	*seconds*	*cu. mm. per second*
120	0.18	180	0.50
150	0.27	60	2.60
180	0.26	90	1.90
180	2.50	120	1.30
60	0.20	180	0.40
60	0.20	210	0.14
		90	0.10

paper. The volume output of blood per second was estimated by dividing the total amount of blood lost, by the bleeding time in seconds.

Similar findings to those in tables 1 and 2 were obtained on normal and thrombopenic dogs.

The bleeding time may be so short as to make it difficult to obtain blood for ordinary cell counts. This is true particularly in cases of chronic myeloid leukemia with high platelet count, following splenectomy and as the patient begins to react from an attack of purpura. The puncture may yield but a single drop, or two very small drops.

Normally there is a certain rhythm in the delivery of blood from a wound in the skin. In the acute stage of purpura, that rhythm may be greatly altered (Fig. 2). A large amount of blood may be followed in the next fifteen seconds by relatively smaller ones and these by larger ones and so on. In certain patients and in dogs just recovering from an acute attack of purpura, the following has been observed: After a profuse flow of blood from a cut there is a sudden stop, no indication having been given of the cessation of bleeding by any gradual diminution in the size of the drops. The bleeding time of the mucous membrane of the mouth in the dog was the same as of its skin (0.5 to 5.5 minutes). The test was performed by making cuts, about 0.25 mm. deep, on the mucous membrane of the upper lip and blotting the blood on filter paper at 15 second intervals. During the acute stage of experimental purpura, the mucous membrane bleeding time was prolonged, but never as much as the skin bleeding time taken simultaneously. In one animal, while the mean skin bleeding time was 10 minutes, the mucous membrane bleeding time was 6 minutes; it never exceeded 9 minutes even when the skin bleeding time was longer than one hour.

When a cut in the skin has bled for a time and stopped, it can be made to start bleeding again by applying negative pressure to it with a suction cup. The amount of negative pressure to make a cut resume bleeding varies not only with the age of the cut but with the efficacy of the hemostasis. In normal dogs, thirty minutes after a cut made for a bleeding time determination had stopped bleeding, it took a negative pressure from 90 to 120 mm. of mercury applied from 10 to 15 seconds to make it bleed again. In experimental purpura, when the bleeding time was slightly prolonged, pressures of 20 to 60 mm. of mercury for periods of 2 to 5 seconds sufficed to make a cut resume bleeding. When the

animal recovered from the attack and the bleeding time became very short, pressures as high as 150 mm. for as long as 30 seconds were sometimes necessary. Perhaps this method will be of some use in indicating a disposition to bleed during or after operations. Clots of blood from individuals with thrombopenic purpura are soft and retract very poorly. It is possible that in the deficiency of these clots lies the reason for their being easily displaced by the lower negative pressure. There was a good correlation between the retractility of the clot and the amount of pressure needed to make a cut bleed. Observations of these changes were made on six animals at various stages of experimental purpura produced by antiplatelet serum. Pressures applied for 10 seconds or less:

Dog 66, female collie. Platelets, 367,000; mean bleeding time, 40 seconds; clot retraction, 2.7 units (normal range for dog, 1.8 to 3.0); bleeding pressure, 100 mm. 1.8 cc. of antiplatelet serum was given intravenously and one hour and thirty minutes after: platelets, 22,000; mean bleeding time, 650 seconds; clot retraction, 0; bleeding pressure, 80 mm. One day after: platelets, 26,000; mean bleeding time, 700 seconds; clot retraction, 0; bleeding pressure, 40 mm. Three days after: platelets, 34,000; mean bleeding time, 690 seconds; clot retraction, 0; bleeding pressure, 30 mm. Four days after: platelets, 70,000; mean bleeding time, 260 seconds; clot retraction, 1.0; bleeding pressure, 120 mm. Six days after: platelets, 235,000; mean bleeding time, 60 seconds; clot retraction, 2.6; bleeding pressure, 130 mm. Nine days after: platelets 560,000; mean bleeding time, 20 seconds; clot retraction, 2.5; bleeding pressure, 115 mm.

MEAN BLEEDING TIME

In careful clinical work it is advisable to do three or more determinations of the bleeding time and obtain a mean from them. One single normal bleeding time is of little significance, particularly if there are evidences of hemorrhagic disease. Instances are not rare of a normal bleeding time from one ear lobe and a prolonged one from the opposite ear. Szecsi[8] thought at least five bleeding times necessary and Roskam[4] insisted on the importance of multiple determinations for an understanding of the factors influencing spontaneous hemostasis and for the pharmacodynamic study of hemostatic substances. In clinical work, however, a single prolonged bleeding time renders further determinations unnecessary and even undesirable for real difficulty may be

experienced in checking the bleeding in some instances. It should not be necessary to continue a determination if it exceeds 25 minutes as little or nothing may be learned by doing it. There is an appreciable loss of blood involved in prolonged determinations and a harmful effect is produced on the patient's mind by seeing an apparently endless flow of blood. The result of the test can be recorded as "25′+." For clinical purposes the bleeding time is considered prolonged whether it is 25 minutes or one hour.

FACTORS INFLUENCING THE BLEEDING TIME

Intravascular. (1) The character of the blood clot and its retractility play an important part in the duration of the bleeding time. The correlation between the degree of clot retraction and the mean bleeding time as found in dogs in various stages of experimental purpura was -0.630 ± 0.04. This was the highest correlation found between the mean bleeding time and any other variable.[9] It should be kept in mind, however, that clot retraction is greatly influenced by the platelet content of the blood. (2) Platelets. In the group of animals just mentioned there was a correlation of -0.424 ± 0.026 between the number of circulating platelets and the mean bleeding time. This figure does not, however, adequately express the relationship between these two variables since the number of circulating platelets does not give a true idea of the available number of platelets being utilized at any given time. (3) The fibrinogen content of the blood. With very low fibrinogen, the bleeding time is generally prolonged. There may be, on the other hand, a prolongation of the bleeding time in the presence of normal fibrinogen values; such was found in our animals with experimental purpura.*

The rate of coagulation of venous blood has little influence on the mean bleeding time. The correlation between these variables as found in the group already mentioned was low ($+0.113 \pm 0.056$) and insignificant. The pressure of the blood within the vessels should conceivably affect the bleeding time. Other factors being normal, a change in pressure perhaps influences the bleeding time

* Unpublished data.

very little. Furthermore, in patients with a prolonged bleeding time, no alterations have been found in the arterial blood pressure. Likewise no significant changes were found in the venous pressure of dogs with experimental purpura.* If, on the other hand, the venous pressure was raised artificially in these dogs, there was not only a lengthening of the bleeding time but an increase in the volume output of blood. The same was observed in patients in the acute stage of purpura.

Vascular. Changes in the vessels are said to, and conceivably do, affect the duration of the bleeding time, yet there is no clear, direct evidence demonstrating such a relationship. Furthermore many of the alterations said to be present in the vessels may be secondary to a thrombopenia. There are, on the other hand, many conditions associated with recognized changes in the vessels in which the bleeding time is not affected, for example, scurvy, hereditary hemorrhagic telangiectasia, Raynaud's disease. Roskam[5] has offered indirect evidence that the vessels are injured in purpura with a prolonged bleeding time. I have investigated the bleeding time in a series of sixteen patients with varicosities in the lower extremities. The tests were performed in the skin adjacent to the varicosities; there were no significant departures from normal. In one patient with extensive sclerotic changes in both upper extremities, cold fingers and a barely palpable pulse, the mean bleeding time on the left and right hands was 30 seconds, with a very low volume output. In this instance, the deficient blood supply to the extremities perhaps accounted for the short bleeding time.

The skin vessels may be collapsed either through pressure of the surrounding tissue or because of an unequal distribution of the blood to the part. In dogs recovering from experimental purpura the skin became very tense and pale; it was then that the bleeding time was short with a low output of blood.

Extravascular. The resistance offered by the tissues surrounding the vessels perhaps plays a rôle in hemostasis. The bleeding time is performed on the skin, and changes in it might affect its duration. In animals with experimental purpura, the skin

* Unpublished data.

becomes loose and deficient in retractility. When a cut is made in it, the edges of the wound gape apart and offer little opposition to the outflow of blood which dissects around the edges, forming a large hematoma. Measurements of the tension and elasticity of the skin have shown a diminution in the acute stage of experimental purpura.[10] The structure of the skin where the cut is made is known to influence the duration of the bleeding time. For example, on certain areas of the soles of the feet only very little bleeding follows a small cut. The skin also plays a part as a close envelope of underlying tissues in checking bleeding. The same might be said to apply to the mucous membranes. An ulcerated mucous membrane is less able to resist or check underlying bleeding than an intact one. One of the methods used for hemostasis, the application of simple pressure, is known to stop bleeding of the purpuric type from skin wounds. This makes it imperative that in doing bleeding time determinations no pressure be applied to the lips of the wound.

THE EFFECT OF VARIOUS AGENTS ON THE EXPERIMENTAL BLEEDING TIME

These observations were carried out in rabbits, by the method of lavage of wounds already described. Approximately eighty determinations were done with each experiment and the mean calculated from them.[3]

Hemorrhage had a tendency to prolong the mean bleeding time and the prolongation was proportional to the quantity of blood withdrawn or lost.[6] Cold (temperatures below 20°C.) and heat (48°C. or above) also prolonged the bleeding time.[3] This fact led Roskam to question the wisdom of cold applications generally employed to stop bleeding. Local injections of perparine, benzol, eupaverine and histamine prolonged the bleeding time; scurocaine definitely shortened it and acetylcholine did not affect it. The serum from animals in anaphylactic stage, a solution of pure pectin, a lung extract, a mixture of prothrombin and thromboplastin did not materially alter the mean bleeding time.[7] An extract made from blood platelets produced a marked diminution when applied locally or injected intravenously.[7] Many of these sub-

stances have an accelerating action on coagulation of the blood yet they were in many instances ineffective as hemostatic agents, some even retarding hemostasis. The following substances, when infiltrated about the wound, had a harmful effect on the bleeding time: lung extract, calcium gluconate 10 per cent, peptone 5 per cent, ergotamine tartrate, horse serum, chloroform serum, gelatin; by intravenous injections only posterior pituitary extract had a definite shortening effect on the mean bleeding time; epinephrine and ephedrine had a very marked retarding effect on the spontaneous hemostasis when injected either around the wound or intravenously; epinephrine did not affect the bleeding time when applied directly to the wound but application of ephedrine definitely prolonged it; intravenous injection of pitressin shortened the bleeding time and that of pitocin lengthened it; direct application to or infiltration of the wound with salt solution, pH = 5, reduced the length of the bleeding time; with a solution of pH = 9, the bleeding time was prolonged.[7]

From these investigations, which it should be remembered were done on animals, it was evident that many supposedly hemostatic agents prolonged the bleeding time instead of shortening it, and that platelet extract, H + ions (pH = 5) and posterior pituitary extract have a constantly favorable effect on hemostasis. The optimum pH for coagulation of the blood is around 7[12] which again indicates the little relation existing between the bleeding time and the coagulation of the blood.

SUMMARY

A device is presented which will produce an incision of approximate uniform dimensions in the skin and give it a graded amount of trauma. The bleeding time is done on the skin and therefore may not necessarily express the hemostatic power of every tissue in the body. One single prolonged bleeding time is of great significance. When normal, multiple determinations should be made. There are quantitative and qualitative variations of the bleeding time both in duration and volume output of blood. The negative pressure required to make a cut resume bleeding after having stopped is lower than normal during acute experimental purpura

and higher than normal after recovery. The bleeding time is influenced by intravascular, vascular, and extravascular factors. There is a high correlation of inverse type between the mean bleeding time and degree of retraction of the blood clot. The correlation between the rate of coagulation of the blood and the bleeding time is very low. Of many substances used as hemostatic agents (some of which are accelerators of coagulation) only platelet extract, H + ions (pH = 5), and posterior pituitary extract had a favorable action on the experimental bleeding time.

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SERODIAGNOSIS OF MALIGNANT TUMORS*

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Very little of worth while material remains after one boils down to essentials the vast literature that has accumulated on the subject of serum diagnosis of cancer during the last thirty-five to forty years. One could not briefly discuss the subject and do justice to the many theoretical and scientific problems that are intimately connected with the immunological and serological aspects of cancer, and I shall review briefly only the practical results of the various diagnostic tests for cancer. When the various tests are analyzed according to the underlying basic principles, they can be divided into nine groups.

(1) *Hemolytic tests.* Principles: (a) Presence of lysin in serum, (b) antihemolytic properties of serum (they are lowest in cancer).

The changes underlying these tests are not specific and the practical results have proved valueless.

(2) *Tinctorial tests.* Principles: (a) Staining of serum by a dye, (b) decolorization of a dye by the serum, (c) development of a color in a mixture of the serum with a colorless compound.

Recent example: Roffo's test.[31] Addition of neutral red to cancerous serum stains it red, while a non-cancerous serum turns yellow.

The reports on the Roffo test show clearly a phenomenon which is encountered in the study of the literature of this subject. A test is very successful and specific in the hands of the author or of those who work with him, while the results of checks by other investigators are most frequently devastating. The results of this group of tests are of no practical value.

(3) *Physico-chemical tests.* Principles: (1) Changes in sur-

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face tension in mixtures of: (a) extracts of tumor tissue with serum (meiostagmin reaction of Ascoli and Izar);[2] (b) of chemical compounds with serum (lecithin, capronic acid, ricinolic acid).

(2) Changes in the electric capacity. It was found increased in malignant tumors (Waterman,[34] Fricke and Morse,[16] Mendeleeff).[28]

TABLE 1

SERODIAGNOSTIC TESTS FOR MALIGNANT TUMORS

(1)	Hemolytic tests
(2)	Tinctorial tests
(3)	Physico-chemical tests
(4)	Precipitation and flocculation tests
(5)	Complement fixation tests
(6)	Cytolytic tests
(7)	Enzyme tests
(7a)	The Fuchs test
(8)	Biologic tests
(9)	Skin tests

TABLE 2

REPORTS ON THE ROFFO TEST

AUTHOR	RESULTS POSITIVE FOR CANCER IN			
	Cancer		Controls without cancer	
	Cases	Result	Cases	Result
		per cent		*per cent*
Members of the Institute of Roffo (Inst. med. exp. Buenos Aires):				
Carranza[7]	140	65	3,067	0.37
Gandolfo[17]	4,282	60	6,718	6.0
Other authors:				
Bajc (Vienna)[3]		50		30.0
Botlin (Belgium)[6]		63		32.0
Carulla (French)[8]		88		81.0

(3) Changes in the concentration of electrolytes in serum. To this group belongs the recently reported method of Links.[25] It is based upon the observation of a relation between the phase of the coagulation of blood and the concentration of certain electrolytes, particularly of calcium and magnesium. Links claims that

he observed changes characteristic for cancer. The physico-chemical methods hold promise of great possibilities although until now no practical results have been achieved.

(4) *Precipitation and flocculation methods.* Here belongs the largest number of diagnostic tests. They are based on colloidal reactions, most of which are not well understood. Some represent a mixture of serological and of chemical principles. The basis of all tests is, changes in dispersion. Flocculation is observed in mixtures of serum with tumor extracts or with various chemical compounds. The selection of the chemical compounds and of the technical procedures in some of the tests are not infrequently an evidence more of the poetic imagination of the authors than of scientific reasoning. Some of the reagents are: Mixtures of sodium vanadate with acetic acid (Bendien test[4]), of Lugol's solution with citric acid and formalin (Botelho[5]) and so forth. All these reactions are based upon non-specific changes due to an instability of the serum proteins, a condition which cancer serums share with serums in many other conditions (pregnancy, tuberculosis, syphilis, etc.) Sachs[33] showed recently that a simple precipitation test with an alcoholic solution of lecithin gives results that are at least as good as those obtained with the most complicated procedures. He also demonstrated how accidental factors may mislead if evaluation of the results is not critical. As shown in table 3 if conclusions had been drawn after completion of the first series, they would have justified a great deal of optimism. The second series indicates the importance of accidental factors inherent in the selection of the material. The location of the tumor seemed to influence the results very considerably.

(5) *Complement fixation tests.* In this group, the investigations of Hirszfeld and of his associate Halber[20] deserve particular attention. They use alcoholic cholesterolized extracts of cancer tissue. Hirszfeld's work has suffered greatly from the attempts of various authors to apply it clinically while the originator of the test emphasized again and again that it is far from being fit for clinical use.

(6) *Cytolytic tests.* Principle: Cancer cells are dissolved by

normal serum and left intact by cancer serum. Best results were obtained by Freund and Kaminer.[11,12,13,14,26,35] The test has suffered from too many false positive results in non-cancerous controls and from considerable technical difficulties in the preparation of suitable cell suspensions.

(7) *Enzyme tests.* They are based upon the principle of Abderhalden's defensive ferments[1] that was originally developed for the diagnosis of pregnancy. The cytolytic and enzyme tests are now only of historical interest as forerunners of the so-called Fuchs test.

TABLE 3

THE SACHS FLOCCULATION TEST FOR CANCER

DIAGNOSIS	NUMBER OF CASES	POSITIVE		QUESTIONABLE POSITIVE	
		Number	Percentage	Number	Percentage
First series					
Cancer	225	119	52.8	18	8.0
Tuberculosis	291	62	20.9	12	4.1
Positive Wassermann reaction	153	23	15.0	10	6.5
Pregnancy	96	6	6.2	1	1.0
Cases without cancer	1,716	128	7.4	34	1.9
Second series					
Cancer	95	43	45.2	8	8.4
Without cancer	52	21	40.4	3	5.8

(7a) *Fuchs test.*[36 to 75] The Fuchs test was developed from the cytolytic test of Freund and Kaminer and from the enzyme test of Abderhalden. The difficulties connected with the estimation of cytolysis in the counting chamber in the Freund-Kaminer test were met by Neuberg[29] who introduced the determination of the non-protein nitrogen in the serum digest. Its increase permitted the recognition of cell digestion. Fuchs found that the serum of normal individuals digested the fibrin of patients with cancer, while the cancerous serum lacked that ability. He later simplified the technic by the use of a precipitate of serum instead of

fibrin. The unknown serum was incubated in test tubes with test material from known cancer serum and from normal serum. The increase of the non-protein nitrogen in the supernatant fluid was determined by means of an extremely sensitive technic which was able to give reliable results up to the second and third decimal fractions of a milligram. An increase of the non-protein nitrogen in the supernatant fluid from the mixture of the unknown serum with the test material of normal serum indicates the presence of cancer, and the diagnosis of absence of cancer is made when an increase is found in the mixture of the test serum with the test material from cancerous serum. The results are estimated by comparison with the amount of non-protein nitrogen in the untreated serum of the patient.

The test does not permit differentiation of carcinoma from sarcoma. Occasionally a decrease of the non-protein nitrogen is observed in tests with the serum from patients with cancer. That signifies a rise of immunity against cancer and is frequently seen following x-ray and radium therapy and after operations. The reaction between the serum of one person and the constituents of the serum of another person takes place not only between cancerous and non cancerous serum, but also between serums of persons free of tuberculosis, syphilis, scarlet fever, etc., and the test material prepared from the serum of persons afflicted with those diseases. On the other hand, the inability of a serum to digest tuberculous, syphilitic, or scarlet fever test material establishes, according to Fuchs the presence of that particular disease in the person whose serum was used.

The first part of table 4 shows the results of the Fuchs test in five patients. The diagnosis is made by comparing the value for the non-protein nitrogen in the plain serum with the values in the mixtures of the serum with the fibrins from different sources. The ability to digest the fibrin as evidenced by a rise of the non-protein nitrogen indicates that the patient is not afflicted with the disease from which the person suffers whose fibrin was used for the test. On the other hand if there is no rise of the non-protein nitrogen in the mixture of a serum with a particular fibrin, that indicates the identity of the conditions in the two persons, from

TABLE 4

THE FUCHS TEST

	MILLIGRAMS OF NON-PROTEIN NITROGEN				
	I	II	III	IV	V
1.0 cc. of serum	**34.528**	**42.336**	**33.678**	**40.476**	**43.228**
1.0 cc. of serum + normal fibrin	**34.520**	44.258	35.342	43.872	47.654
1.0 cc. of serum + cancer fibrin	36.224	**42.342**	35.628	43.986	**43.230**
1.0 cc. of serum + syphilis fibrin	35.986	44.622	**33.670**	44.032	45.994
1.0 cc. of serum + tuberculous fibrin	36.486	45.042	36.202	**40.480**	**43.222**
Result: Cancer	−	+	−	−	+
Result: Syphilis	−	−	+	−	−
Result: Tuberculosis	−	−	−	+	+
	Negative	Cancer	Syphilis	Tuberculosis	Cancer and tuberculosis

IMMUNOLOGICAL RESPONSE IN A CARCINOMA OF THE BREAST

	MILLIGRAMS OF NON-PROTEIN NITROGEN				
	A	B	C	D	E
1.0 cc. of serum	**39.760**	**44.322**	41.546	39.228	40.692
1.0 cc. of serum + normal fibrin	41.032	46.246	41.148	38.824	39.578
1.0 cc. of serum + cancer fibrin	**39.756**	**44.328**	40.796	36.246	38.854
Result	Positive	Positive	Positive immune	Positive immune	Positive immune

(A), before irradiation; (B), twenty-three hours after irradiation; (C), sixty-five hours after irradiation; (D), 148 hours after irradiation; (E), eleven days after irradiation.

one of which comes the serum (the unknown) and from the other the fibrin (with the known disease). In the first column in table 4, the values for the non-protein nitrogen are identical in the plain

serum and in the mixture of that serum with fibrin from a known healthy person, while in mixtures with fibrin from patients with cancer, with syphilis, and with tuberculosis, an increase is noted. That means that the tested serum comes from a person without cancer, syphilis, or tuberculosis. In the second column there is no increase in the mixture of the serum with the fibrin of a known cancer patient, as compared with the untreated serum. That indicates the presence of cancer. The interpretation of the results in the three other columns is self explanatory. The second half of the table shows the so-called immunity reaction. Beginning with the third column there is a decrease of non-protein nitrogen in the mixtures of the serum with cancerous fibrin. That result indicates an immune reaction, as a result of which the combinations of the antigen with the antibody form large molecular aggregates that are precipitated and therefore do not appear in the protein-free filtrate. According to Fuchs that phenomenon is analogous to the decrease of non-protein nitrogen in mixtures of diphtheria toxin and antitoxin as reported by Ramon.

While one is naturally inclined to approach such claims with a great deal of caution, one cannot ignore the fact that until 1934, 5000 tests were carried out according to this procedure with about 92 per cent to 94 per cent of correct results and with relatively few false positives. The test was uniformly negative in cancer of the esophagus and of the larynx. The test offers great technical difficulties. A special apparatus is required for the determination of the non-protein nitrogen and a special colorimeter for its estimation. The test was not confirmed by Yokota[75] and Van der Scheer,[73] but most other authors in different countries have found it surprisingly reliable. The great difficulty lies in the very minute differences in the values of the non-protein nitrogen that determine the positive or negative result. They lie within the limits of the error of the usual methods (± 3 per cent). The apparatus of Fuchs which permits an extremely accurate determination is at present quite expensive.

A recent modification by Chrometzka and Gottlebe[63] seems to offer help because it eliminates a source of error by increasing the

differences in the values of the non-protein nitrogen. That is accomplished by using a protein free filtrate for the test. The same authors developed also three simplified modifications of the Fuchs test, a qualitative precipitation method and a qualitative colorimetric method, and a quantitative colorimetric method.

Caspary found recently an increase in the values for non-protein nitrogen following removal of the calcium ions.

(8) *Biologic tests.* Space does not permit the discussion of the very important contribution of Ferguson[10] and others, according to which some tumors can be detected by the demonstration of an increase of anterior pituitary hormone by means of the Aschheim Zondek test. The other tests in this group are non-specific and unreliable. The so-called urochrome reaction of Davis[32] is interesting and very simple to carry out. Urine is slightly acidified and extracted with ether. The extract is boiled and in cases of cancer a reddish color appears, a yellow to brown color indicates a negative result. Up to 95 per cent positive results were reported in established cases of cancer and up to 31 per cent in cases free of cancer.

(9) *Skin test.* Attention was attracted to a skin test of Freund and Kaminer.[9,15,21,22,23,30] They plant intestinal contents of patients with cancer upon a special culture medium (fat milk). An ether extract of the growth and of the culture medium is prepared accordingly and used for intracutaneous injections. A comparison of results by different authors mainly in Austria and in Germany gave the following figures: of 488 cases of cancer 91 per cent gave positive results, 3 per cent questionable, and 6 per cent false negative results. In 443 cancer free control cases, the results were: 73 per cent negative and 17 per cent false positive. The originators of the test explain the rather high percentage of false positives as indicating the high sensitivity of their test. It may detect a tendency towards carcinoma.

The Gruskin skin test[18,19] uses embryonal tissue for intracutaneous injections. The very favorable results reported by Gruskin are contrasted sharply by McFarland and associates[27] who found the test to be wanting and not helpful when most needed.

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BONE MARROW STUDIES IN GLANDULAR FEVER (INFECTIOUS MONONUCLEOSIS)*

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Glandular fever (infectious mononucleosis) is described as an acute, infectious-like disease, the etiology of which is as yet undetermined. The onset is acute, beginning usually with loss of appetite, weakness and sore throat. The symptoms are rapidly intensified, and fever, lymphadenopathy and mononuclear leukocytosis supervene. Remaining at the peak of severity for about two weeks, these symptoms gradually subside. The disease has occurred in small sporadic epidemics and most commonly in young adults who are hospital employees, such as internes, nurses and attendants.[3] It has been reported, however, in persons under ten and over seventy years of age.

The condition of the throat has often been described as a true Vincent's angina and stomatitis. Weakness varies in intensity but is usually sufficiently severe to force the patients to remain in bed. The temperature fluctuates, being normal in the morning and high in the late afternoon and evening. The submaxillary and posterior lymphatic nodes are almost invariably enlarged; those in other parts of the body, including the spleen, are sometimes palpable. This hypertrophy, simulating that in acute lymphatic leukemia and Hodgkin's disease, is caused by a diffuse hyperplasia of the germinal centers with consequent infiltration of the parenchyma by both mature and immature lymphocytes. The total leukocyte count varies between fifteen and thirty thousand. It has been rarely found under fifteen thousand and

*Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists, held at Atlantic City, New Jersey, June 7 to 9, 1935.

never over thirty thousand. The leukocytosis is due to an absolute increase in the number of lymphocytes; there is an accompanying relative decrease in granulocytes. While the majority of lymphocytes is composed of adult forms, the remaining cells are peculiarly immature.[1] The lymphocytes have a lobulated nucleus which is somewhat kidney-shape in outline and stains a deep violet color; their cytoplasm is foamy and vacuolated. The immature lymphocytes studied have been from blood smears only.

No record of bone marrow study has been found in the literature pertaining to glandular fever. Microscopic study seems to have been limited to the blood.[3] In isolated instances biopsy material from one or more of the involved lymphatic nodes has been studied, but heretofore the blood has been the chief diagnostic criterion. This paucity of data is probably due to the fact that the disease runs a self-limited course; unless disturbing complications enter, patients afflicted with it are not sick enough to be hospitalized. Since the blood is rarely examined except at a hospital, the disease is usually not suspected. On the other hand, hematological studies are performed as a matter of course on people working in hospitals even though they are only slightly ill. These circumstances can account for the fact that the disease has been discovered more often among these people. Finally, the discovery of one case stimulates a search for others and they are not infrequently found. While these factors may seem unimportant, they must nevertheless be considered in evaluating the belief that glandular fever is of infectious origin and occurs in epidemic form. A study of the bone marrow may help considerably in revealing the nature of the disease. It is with this thought that the following abstracts of case records are presented.

CASE 1*

(1) A white, unmarried, female student nurse, nineteen years of age, was admitted to the Worcester Hahnemann Hospital complaining of weakness, fever, sore throat, and tender swellings in the neck. For two months prior to

* For the clinical examination and record of this patient, I am indebted to Dr. Gerald Shelby of Shrewsbury, Massachusetts.

admission she had lost five pounds in spite of her earnest attempt to gain weight. While on duty she tired easily and became generally weaker even after sleeping several hours more each night than had been her habit. Three days before admission she began to feel chilly and feverish. The day before admission she developed a sore throat and a non-productive cough. She perspired profusely. On the morning of admission she noticed small and tender swellings on the left side of her neck. She reported her illness to the office and was admitted as a patient.

She was found to have an acute Vincent's pharyngitis and tonsillitis; moreover, all the cervical lymphatic nodes, particularly those of the left posterior chain, were enlarged and tender. The spleen was not palpable and there were

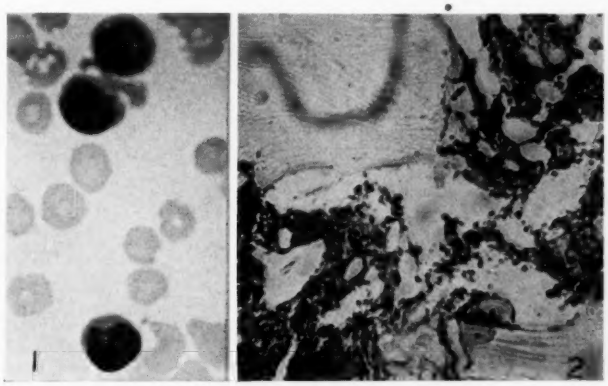

FIG. 1. PHOTOMICROGRAPH OF A BLOOD SMEAR FROM CASE 1

Taken Nov. 29, showing the cells of glandular fever. ×1000. Wright's blood stain.

FIG. 2. PHOTOMICROGRAPH OF SECTION OF STERNAL BONE MARROW

Biopsy from case 1, Nov. 29. ×100. Hematoxylin-eosin stain. Note the cellular replacement of the marrow spaces.

no discernible intradermal or intramucous hemorrhages. Her oral temperature was 99.8°F.; the pulse rate was 100 per minute and the respiration rate, 20. The hematological examination on admission showed a hemoglobin content of 70 per cent as determined by the Tallquist method; there were 4,290,000 erythrocytes and 10,400 leukocytes per cubic millimeter of blood. The differential count was: neutrophils, 24 per cent and lymphocytes, 76 per cent. Three days later, the sixth day of acute illness, the number of leukocytes increased suddenly to 18,750, of which 13.5 per cent were neutrophils and 87.5 per cent were lymphocytes. Of these latter cells many were immature, such as are found in the blood in cases of glandular fever (fig. 1). The platelet count was normal. The heterophile antibody test was not performed.

It was thought desirable to rule out acute lymphatic leukemia in order to better prognosticate the outcome. A sternal bone marrow biopsy was considered the most promising aid. The literature was extensively searched for any record of bone marrow findings in cases of glandular fever, but none was found. In lymphatic leukemia, however, the bone marrow is massively infil-

TABLE 1

BLOOD EXAMINATIONS. CASE 1

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	NEUTROPHILS	MONONUCLEAR LEUKOCYTES	REMARKS
	per cent	*millions*		*per cent*	*per cent*	
11/25	70	4.29	10,400	24.0	74.0	
11/28			18,750	13.5	87.0	240,000 platelets. Many "glandular fever cells" found.
11/29			19,600	12.7	87.0	10 per cent peroxidase positive cells.*
11/29					84.5**	More than half of the lymphocytes were immature, the remainder were an adult type. 15.5 per cent were erythroblastic and myelogenous cells.
11/30	70	4.24	19,200	10.7	89.0	No change in the differential count.
5/20***	80	4.3	8,000	67.0	33.0	No immature cells found.

* The peroxidase stain used was the so-called, "Dopa" stain[2] which is the trade name for l-dioxyphenylalanin sold by Hofmann-LaRoche & Co.

** Sternal bone marrow.

*** Records of the blood studies made during the interval are deleted because the results do not differ materially from those of November 30, except for the total leukocyte count which became gradually decreased.

trated with lymphocytic cells. It was reasoned therefore, that if glandular fever is infectious-like in nature, the bone marrow should not be so infiltrated. The biopsy was performed the seventh day of the patient's acute illness.

The marrow smears contained 84.5 per cent of lymphocytes. More than half of these were immature and identical with those found in the peripheral blood. The other lymphocytes were adult in type. The remaining 15.5 per cent of the cells belonged to the erythroblastic and myelogenous groups. The marrow spaces on histological sections were filled with these cells (figs. 2 and 3).

The subsequent course of the patient's illness was typical of glandular fever. Although the patient was symptom-free one month after the acute onset of the disease, her blood did not return to normal, morphologically, until almost six months later. Table I contains only the essential details of the blood counts.

Today the patient is married and expects to be delivered in about four months of her first child. She has had no return of her former symptoms.

Why, in glandular fever, should the bone marrow be infiltrated with lymphocytic cells? Does it not suggest that the infiltration is similar to that of a leukemia? In table 2 is compared the essential features of the findings in glandular fever and acute lymphatic leukemia. Are not the real differences merely of

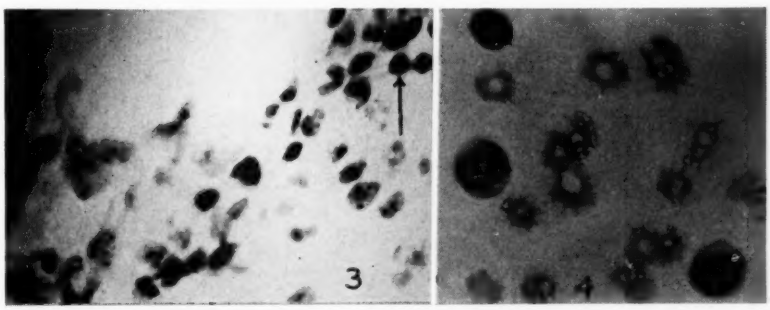

FIG. 3. HIGH POWER MAGNIFICATION OF A FIELD IN FIGURE 2. ×1000
Arrows point to "glandular fever" cells. Compare with figure 1

FIG. 4. PHOTOMICROGRAPH OF A BLOOD SMEAR FROM CASE 2
Taken the day after hospital entry (the seventh day of her acute illness). ×1000. Wright's blood stain.

degree? In the former, the patients are not seriously ill, while in the latter they invariably die. In acute lymphatic leukemia the clinical symptoms are intensified as compared with those of glandular fever. So far then, the two diseases bear striking similarities except for prognosis and severity. It is possible therefore that the two are closely related, and that consequently an illness beginning as glandular fever might terminate as acute lymphatic leukemia. The case record of the following patient may be illustrative of this point.

TABLE 2

COMPARISON OF THE ESSENTIAL FINDINGS IN GLANDULAR FEVER AND ACUTE LYMPHATIC LEUKEMIA

	GLANDULAR FEVER	ACUTE LYMPHATIC LEUKEMIA
Occurrence	Children and young adults usually.	Children and young adults usually.
Etiology	Unknown. Usually follows or is accompanied by an attack of Vincent's stomatitis or tonsillitis.	Unknown. Usually follows or is accompanied by an attack of Vincent's stomatitis or tonsillitis.
Signs and symptoms	Intermittent fever. Malaise, weakness and headache. Cervical adenopathy. Spleen may become palpable and somewhat tender. Bleeding lesions of the mouth. Transient hematuria. Glandular enlargement and blood changes usually persist for months after the disappearance of the clinical symptoms.	Intermittent fever. Malaise, headache, and extreme prostration. All superficial lymph glands are enlarged. Moderate enlargement of the spleen and liver. Bleeding lesions of the mouth or gastrointestinal tract. Hematuria with casts and albumen.
Blood findings	No significant change in hemoglobin or number of erythrocytes. Color index is usually normal. The leukocyte count varies from 3,000 to 35,000	Hemoglobin is lowered, may be below 20 per cent. Erythrocyte count very low. The color index is usually higher than one. The leukocyte count is often below 10,000 at first. Leukopenia may persist throughout the entire course of the disease (aleukemic phase), otherwise the leukocyte count rapidly rises to 100,000 or more.

TABLE 2—*Concluded*

	GLANDULAR FEVER	ACUTE LYMPHATIC LEUKEMIA
Blood findings...	The predominating cell is the typical immature lymphocyte with the indented nucleus, and may be accompanied by other immature forms to about 90 per cent.	The cells of the lymphocytic series predominate, 90 to 99 per cent. All stages of immature lymphocytes are found.
	There is a relative neutropenia.	Blood platelets and neutrophiles are decreased relatively.
Pathology.....	Lymphatic nodes differ from acute lymphatic leukemia only in the degree of hyperplasia.	There is a diffuse infiltration of all tissues by the lymphatic cells.
	No bone marrow studies have ever before been reported. Now is reported as infiltrated by the immature lymphocytes.	The bone marrow is likewise infiltrated together with an aplasia of the myeloid and erythroblastic cells.
Prognosis.....	Is in itself not serious and not fatal. Patients always recover.	Most cases are rapidly fatal with death occurring from a few days to a few weeks. Remissions do occur rarely but death always results.

CASE 2*

An unmarried, white saleswoman, fifty-five years old, was sent to the contagious hospital with a diagnosis of diphtheria. She complained of a sore throat, headache, fever, and malaise of four days duration. For four weeks prior to the onset of her symptoms, she had become increasingly fatigued while working. About one week before admission she had grown so weak that she was forced to remain in bed. Three days later she developed a sore throat and headache and began to feel feverish. Her symptoms increased in severity. Two days later she expectorated a small quantity of bright red blood. This circumstance made her call her family doctor, who immediately had her transferred to the hospital.

At the institution no diphtheria bacilli were found. She did have, however, an acute Vincent's pharyngitis and tonsillitis. The blood pressure was 130/58;

* For the clinical examination and record of this patient, I am indebted to Dr. Constance Kaliris of Worcester, Massachusetts.

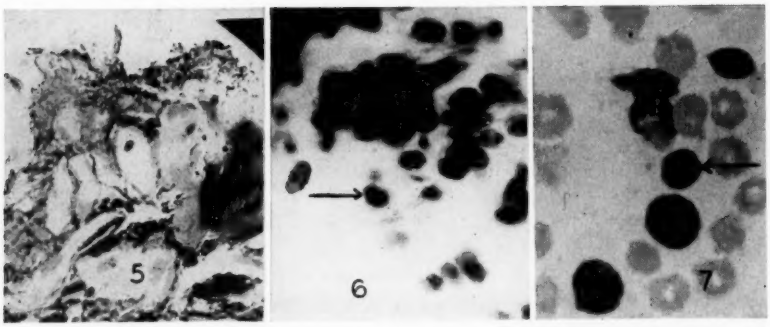

FIG. 5. PHOTOMICROGRAPH OF SECTION OF STERNAL BONE MARROW

Biopsy from case 2, taken the thirteenth day of her acute illness. ×100. Hematoxylin-eosin stain. Compare with figure 2.

FIG. 6. PHOTOMICROGRAPH OF A HIGH POWER MAGNIFICATION OF FIGURE 5. ×1000

Compare with figures 3 and 4

FIG. 7. PHOTOMICROGRAPH OF A BLOOD SMEAR TAKEN FROM CASE 2, THE MORNING PRIOR TO DEATH

The diagnosis had been changed to acute lymphatic leukemia. ×1000. Wright's blood stain.

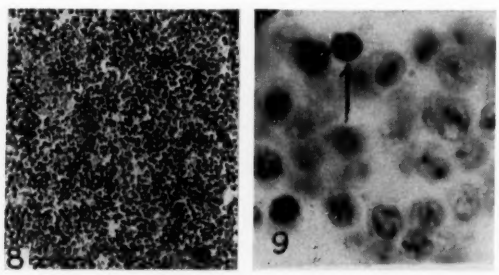

FIG. 8. PHOTOMICROGRAPH OF SECTION OF FEMORAL BONE MARROW FROM CASE 2, REMOVED AT NECROPSY. ×100

Hematoxylin-eosin stain

FIG. 9. PHOTOMICROGRAPH OF A HIGH POWER MAGNIFICATION OF FIGURE 8

Arrow is pointing to one cell which is identical with those found in the blood and biopsies of bone marrow. ×1000.

oral temperature, 101.8°F.; pulse, 110 and respirations, 24. The cervical lymphatic nodes were easily palpable and tender. The hemoglobin content of the blood was 45 per cent; the erythrocytes numbered 2,270,000 per cubic millimeter, and the leukocytes 20,000, of which 57 per cent were lymphocytes, many being identical with those found and described in the blood of the first case (fig. 4). Permission for a sternal bone marrow biopsy was not obtained until the patient had been in the hospital six days, the 13th day of her acute illness. During this time the blood had been examined daily but showed no material change. At the time that the sternal bone marrow biopsy was performed, her hemoglobin was 45 per cent; there were 1,110,000 erythrocytes, 156,000 platelets, and 23,600 leukocytes per cubic millimeter. The differential count showed 9 per cent neutrophils, 17 per cent adult lymphocytes, and 74 per cent immature lymphocytes identical with those seen in the previous blood smears. In the bone marrow 76.5 per cent of the cells were immature lymphocytes, 20.5 per cent were undifferentiated "blast" cells, and 2 per cent were erythroblasts. The marrow spaces were filled with these cells and in about the same proportion (figs. 5 and 6). The heterophile antibody test was not performed. It is readily seen that up to this time the clinical and laboratory pictures of the patient's illness were almost identical with those of the first patient.

On the day following the biopsy the blood leukocyte count suddenly rose to 91,800. This sudden and enormous increase in the number of leukocytes may have been correlated with the high percentage of undifferentiated "blast" cells in the bone marrow on the previous day. In fact it was on this day that lymphoblasts were found in the patient's blood smears for the first time. The number of lymphoblasts increased in ratio, daily. The number of leukocytes then increased on successive days to 143,650, 217,600, and 392,000 respectively, the last being on the day she died, the 17th day of her acute illness (fig. 7). On account of the steady rise in the leukocyte count and the rapid increase in the number of lymphoblasts, the antemortem diagnosis was changed to acute lymphatic leukemia. An autopsy performed an hour and a half after death confirmed it. The peculiar, immature lymphocytes characteristic of glandular fever which were found in the blood smears were also present in the bone marrow removed at necropsy (figs. 8 and 9).

DISCUSSION

The first patient had glandular fever. The examination of her sternal bone marrow revealed the marrow spaces filled with typical immature lymphocytes found in the blood in this disease. The illness of the second patient began in a manner almost identical with that of the first and it remained the same for thirteen days. The sternal bone marrow findings were substantially the same as those in the first case. On the fourteenth day, the

disease of the second patient suddenly developed new characteristics which made it necessary to change the diagnosis to acute lymphatic leukemia. This latter disease killed her. In view of these facts there is some basis for stating that the second patient was ill first with glandular fever and later with acute lymphatic leukemia.

SUMMARY

Case abstracts are presented of two patients suffering from a disease initially symptomatic of glandular fever. The disease of the first patient ran through its usual course and outcome while that of the second patient suddenly developed all the characteristic symptoms of acute lymphatic leukemia and died. Postmortem examination confirmed the latter diagnosis.

Investigations of the sternal bone marrow in both cases and during the earlier stages of the illness showed almost identical pictures, that is, the marrow spaces were filled with both immature and mature lymphocytes.

These findings suggest that glandular fever may really be an abortive, benign form of acute lymphatic leukemia.

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METHOD OF TEMPORARILY PRESERVING FRESH FROZEN SECTIONS STAINED WITH POLYCHROME METHYLENE BLUE

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In laboratories of surgical pathology the polychrome methylene blue stain is most widely used in the diagnosis of tumors. This stain is used on frozen sections of fresh tissue. There have been several objections to its use, the most common one being that it cannot be preserved for more than one or two hours at most. This is a disadvantage, because occasionally one might wish to compare the fresh frozen section stained with polychrome methylene blue with fixed sections stained with hematoxylin and eosin.

In this laboratory during the last few months it has been possible to preserve this stain satisfactorily for several days and even for several weeks. The method employed is, as soon as the diagnosis is arrived at and before the mounting fluid (glucose or water) has evaporated, to paint around the edge of the coverslip with a clear, quick-drying lacquer.* This serves to prevent evaporation of the mounting fluid and to seal it. The blue will of course ultimately (after a few days or weeks) go into solution in the mounting medium; but fixed sections have by that time been prepared and examined. This means of preserving fresh sections is very valuable in teaching graduate students as it allows adequate comparison of the changes in the cells due to fixation and also to changes in architecture, which frequently occur.

The use of a clear, quick-drying lacquer is not limited to fresh frozen sections, as all microscopic preparations mounted in balsam, or some modification of this mounting medium, can be sealed with the preparation. It is also useful in preserving fat stains by surrounding the cover glass, thus preserving the section indefinitely. "Duco" has been used in the laboratory of Pathologic Anatomy as a rapid seal for many of the microscopic preparations, particularly those to be used either as lantern slides or in the microscopic projector.

*Clear "Duco", which is manufactured by the E. I. Dupont de Nemours Company, has been found to be the most satisfactory lacquer for this purpose.

A NEW STAIN FOR CONNECTIVE TISSUE, MUCIN, AND ALLIED SUBSTANCES

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Some years ago a dye manufacturing company* kindly furnished the Section of Pathologic Anatomy of The Mayo Clinic samples of dyes which might be used in staining tissues. One of these, called Orange S, has given rather useful contrasts in combination with various staining methods. Details of the technic are as follows:

A. For staining connective tissue and mucin:
   1. Fix the tissue in formalin; embed in paraffin, and cut sections about 8 microns thick. Mount sections on slides; dry in oven at 36°C. for six to twelve hours, and remove paraffin in the usual manner.
   2. Stain for half an hour in 75 cc. of saturated aqueous solution of picric acid to which 5 cc. of glacial acetic acid has been added.
   3. Wash slide thoroughly in water.
   4. Stain for ten minutes in alum-hematoxylin solution to which 10 drops of staining solution of Orange S has been added. To prepare this solution of Orange S, first make a stock solution by dissolving 1 gram of Orange S in 200 cc. of 95 per cent alcohol and 10 cc. of glacial acetic acid; allow this solution to stand for one week or more. To make the staining solution, add 50 cc. of the stock solution to 25 cc. of absolute alcohol.
   5. Wash slide ten minutes in water.
   6. Stain for twenty minutes or more in alum-lake carmine solution. Make a fresh solution each time by adding 2 grams of alum-carmine and 0.5 gram of aluminum chloride to 20 cc. of distilled water. Heat these slowly until deep red, then add 100 cc. of 95 per cent alcohol and 80 cc. distilled water, filter this solution before using.
   7. Wash slide quickly in water.
   8. Stain for five minutes in 200 cc. of a saturated aqueous solution of picric acid to which 20 cc. of a 1 per cent aqueous solution of indigo carmine has been added.

* E. I. DuPont de Nemours and Company, Wilmington, Delaware.

9. Wash slide quickly in water.
10. Dehydrate in 95 per cent methyl alcohol, acetone, and clear with xylol; and cover slip with Canada balsam.

Mucin is stained brilliant red; cartilage and hyaline substances are stained bluish-red; fibrillar and collagenic connective tissues are stained grass-green; smooth muscle is stained olive green; and striated muscle is stained yellow.

B. For staining amyloid:

1. Cut paraffin sections 8 microns thick; dry in oven over night; remove paraffin in xylol, pass through the alcohols to water, and dry for twenty-one hours in an oven at 36°C.
2. Stain for five minutes in a solution of Harris hematoxylin. After place slide in steaming hot carbol fuchsin for five minutes.
3. Wash slide in tap water.
4. Decolorize quickly in acid alcohol (1 per cent hydrochloric acid in 70 per cent alcohol).
5. Wash slide for ten minutes in running water.
6. Stain for five minutes in picro-indigo-carmine solution.
7. Stain for one minute in the staining solution of Orange S.
8. Decolorize in absolute alcohol; control this procedure with the microscope.
9. When components are sufficiently differentiated, wash specimen in clean alcohol; clear in carbol xylol and xylol; and cover slip with Canada balsam.

Amyloid is stained greenish-gray; connective tissue is stained green; erythrocytes are stained yellow; and nuclei are stained reddish-brown.

EDITORIAL

The Practitioner of Laboratory Medicine

How shall the practitioner of laboratory medicine look at himself?

There are two groups of individuals in the modern medical world—those who prepare for a decision, those who make and use it. The former is frequently called the clinical pathologist but his duties are broader than this term implies and are best covered by the term practitioner of laboratory medicine. His place is well enough known to distinguish him from the so-called pure clinician. It is rare that the qualities of these two are present in the same individual.

Followers of the clinical laboratory calling have done well and they have used their training and their dexterity to develop a handicraft of inestimable value. Because certain of them have had unusual opportunities or possess conspicuous qualities, these have approached the second group in that they are able to interpret and use the product of their handicraft. They seldom have or can obtain all the requirements essential to the calling of the clinician since they must keep constantly in mind the developments of their handicraft, today a Herculean task. None the less, they have ofttimes assumed the position of dictatorship because they have been the preparers of essential information. Many of them have perhaps assumed arrogantly that since they possess the keys to unlock the secret of a problem, they can enter and perform all.

This is, however, not entirely the reason for the evolution of their calling because many of the pure clinicians rely upon them, a dependence somewhat due to ignorance, somewhat from inadequate information but very often from laziness in failing to keep abreast of modern interpretations. However, many practitioners have passed through the laboratory and entered the group that

decides and uses. Such a group needs but little of the words of of the preparateurs but requires their deeds.

Inevitably every man will know one or two things better than many things so that the development of special knowledge and application occurs in calling of the laboratory man as in any other. The applicability of special knowledge within the groups varies directly with the familiarity and discretion in other branches. The chemist is a better advisor in pneumonia if he be informed of the microbial nature of the disease. The pathologist yields more information when he is familiar with the anatomy and chemistry of the body. The practitioner of laboratory medicine is valuable to the degree that he can assemble all the features essential to the problems of his calling. He truly must be familiar with more things than any other of the followers of specialties in the medical fraternity. He must have discretion in every subject —biology, physiology, anatomy, chemistry, hygiene and to be modern, unfortunately, business. Speaking acquaintance with physical diagnosis and therapeutics will do no harm.

Development of such an existence would render impossible that the practitioner of laboratory medicine should be a pure technician. The need of other hands, enforced by the pressure of problems today, has made imperative non-medical assistance, the technician, but no amount of this can relieve the simplest examination of its value when properly interpreted after the responsible officer shall have trained those hands to unfaltering accuracy. These assisting hands may be attached to an individual with an academic degree but not to a head of broadest judgment, not to the experience that permits a word of interpretation. Many a doctor in clinical pathology cannot be considered a practitioner of laboratory medicine. Failure to evaluate this has often lead to the disproportionate self-opinion of some who titrate antigen or note the reduction of iodine solution.

We thus come to an inevitable conclusion that there are possibly three groups instead of two: the instructed preparateurs, the professional man in charge of them, who instructs and who interprets, and the last group, the employers of this information. The question naturally arises as to the difference between the

second and third group and one may truly wonder if a real separation be necessary.

We are faced shortly with the plan of the adoption of arbitrary and temporary standards whereby there shall be presented to the world a group of individuals who may, with the approval of this self-appointed group, state that they are so approved. Shall they be handiworkers or shall they be interpreters? Since the former might well, and indeed should, work for the latter, which is the responsible party? What shall be the criteria? Shall the value of interpretation be estimated by those who recommend, or by those who apply and finally judge? Can a self-appointed board do more than establish methodic standards? Must they of themselves decide that they are worthy of qualification or shall the people who can evaluate their significance, accept of their results and indicate their approval?

It would appear that we have now before us the alternative of drawing standards that will embrace the technical training of the handiworker and the judgment of the experienced, or to ask for the approval of recognized practitioners of laboratory medicine from established organizations of those who use our work and thoughts. There is reason to think that the accredited physicians making up the latter bodies would welcome into their midst the practitioners of laboratory medicine who could prove that their special training entitles them to be co-equal consultants. Thus only will the practioner of laboratory medicine become a true consultant.

HERBERT FOX

NEWS AND NOTICES

The following communication has been received from Dr. Kano Ikeda, St. Paul, Minnesota:

Recently a letter was circularized among the superintendents (not the pathologists) of hospitals throughout the United States, by the director of a commercial school for laboratory technicians in which he calls their "attention to an innovation in the conduct of the courses" offered at his institution. This innovation calls for a "from four to six months' *interneship* in an accredited hospital" for the graduates of the school who have completed an eight months' course in laboratory technic. The director invites the support of the hospital superintendents to this proposal which would be tantamount to the endorsement of his private commercial enterprise and its policies on the part of the co-operating hospitals. The idea of "interneship" for laboratory technicians is not new; it has been advocated by another well advertized commercial schools for technicians during the past several years. It is an attempt on the part of these schools to supplement the inherent deficiencies in their instruction.

I wish to call the attention of the members of the American Society of Clinical Pathologists (which has pioneered in the organized efforts to elevate the standard of laboratory service and of the qualifications of technicians), to this widely circularized advertisement and urge them to study the proposal and its probable effects upon the laboratory service of the hospitals, particularly where no full time supervision of the pathologist is available. Grossly misleading is the sentence that the director is "offering you (the superintendent) an opportunity to get good workers and at the same time to assist in uplifting the character of work to be done in our modern hospitals." This is a particularly dangerous statement when one realizes that many of the "accredited" hospitals (by the American College of Surgeons) likely to take advantage of this plan are small institutions perhaps with only one technician in charge of the laboratory, with or without the actual supervision of even a part time pathologist. Furthermore, in this attempt may be seen a scheme of sidetracking the minimum requirement of the A. S. C. P. which demands, among others, a training period for laboratory technicians of not less than twelve months under a recognized pathologist.

Tempting as it undoubtedly is to the hospital administrator and plausible as it may seem at first thought to the busy laboratory director who is usually handicapped by shortage of technical assistance, the proposition appears to me to be a thoroughly pernicious practice which, if encouraged to exist, will deal a

severe blow to the constructive work which is being carried on by the A. S. C. P. through its Board of Registry.

The whole-hearted support for the program which calls for voluntary regulation of the methods of training and of the qualifications, of laboratory technicians, according to the minimum standards, under the sponsorship of the A. S. C. P., is urgently needed to-day more than ever before.

The following medicolegal items will be of interest to clinical pathologists: during the past year a new California law requires the licensing of clinical laboratories by the State Board of Health and makes it unlawful to conduct a clinical laboratory except under the direction of a clinical laboratory technician licensed by the State Board of Health or a licensed physician or surgeon. Another law regulates the production and distribution of serum, vaccines, bacterial cultures, and viruses. An Oregon law provides for the registration and regulation of laboratories in which human and animal body fluids, secretions, or excretions are examined for the presence of an infectious agent. Antivivisection bills of various kinds were rejected in New York, Wisconsin, and California, some of which would have virtually prohibited experimental work in these states.

Fifteenth Annual Convention A. S. C. P.

According to a letter from the Secretary, the Fifteenth Annual Convention will be held May 8, 9, and 10, in Kansas City, Missouri. Although it is too early to furnish a tentative program, it is clear that there will be many papers of great interest to all clinical pathologists. On the two days preceding the meeting, a seminar will be held in Kansas City. One day will be devoted to hematology under the direction of Dr. Hal Downey and the other day will be devoted to a study of several groups of tumors under the direction of Dr. R. H. Jaffé. One afternoon of the convention will be devoted to a symposium on medicolegal work.

BOOK REVIEWS

The Cerebrospinal Fluid and Its Relation to the Blood. A Physiological and Clinical Study. By Solomon Katzenelbogen. PP. xx + 468, 1935. Baltimore: The Johns Hopkins Press: $5.00.

This valuable monograph consists of two major divisions; the first three chapters dealing with the cerebrospinal fluid as such, its origin, mode of formation and circulation, and the remaining seventeen chapters dealing with the physico-chemical constitution of the fluid as compared with blood in physiological and pathological conditions. The author remarks that "if neither 'secretion' nor 'dialysis' can be applied to the mechanism of cerebrospinal fluid formation, then the term 'physiological or biological permeability' appears to present an adequate expression of the viewpoint that would be a compromise."

Approximately a third of the book is devoted to a thorough discussion of the barrier function and here one finds tests given in detail by which one may test this important function but methods of testing the chemical contents of the cerebrospinal fluid are not given.

The book is made up principally of discussions of the literature, but the author's own experiments and observations covering a period of more than a decade are also cited. For convenience, numerous tables in the text summarize the important findings of previous authors, and at the end of each chapter one finds a short summary clearly setting down the salient points in the chapter. Each individual substance identified in the cerebrospinal fluid is treated in a separate chapter and the significance of variation in the substance is given specific discussion. That the author has combed the literature thoroughly is manifested not only by the text but by approximately 900 references which are appended. Groups of men who should have the book handy for references are neurologists, psychiatrists, physiologists, pathologists, and biochemists.

Immunity. By N. P. Sherwood. PP. 608, 1935. St. Louis: C. V. Mosby Co. $6.00.

This is a book intended as a text for medical students, and is written in a style intended to meet the needs of undergraduate classes. The chapters are subdivided into myriads of short sections, each with a bold face side heading. A large part of the writing is in the form of definitions and statements of facts, without much discussion. There is an especially good chapter on blood groups. Standard technics are given for performing various tests and experiments.

The author reviews in detail the work of Kolmer on the Wassermann test and supports his results. He considers that the flocculation tests are in a state of confusion, which might have been somewhat cleared for him had he been able to include the recent report of the committee appointed by the Surgeon General of the United States Public Health Service. There are extensive lists of references at the end of each chapter and several color plates, the cost of two of which, number 4 and the one containing figures 20 and 21, could well have been saved. The text will be liked by medical students since they will be able to find quickly the answers to examination questions in this subject.

Clinical Diagnosis of Diseases of the Mouth. By Louis V. Hayes. PP. xviii + 461, 1935. Brooklyn: Dental Items of Interest Publishing Co. $7.50.

This book is primarily designed for dentists and physicians who have the problem of diagnosing and treating lesions of the mouth. The book is more of an atlas than a text for it has 353 illustrations, mostly of a clinical nature. A few of these illustrate the histopathology of the lesions. A long list of references is included in the book.